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[GANN, Vol. 43; Dec., 1952]

ON THE LIVER ASPARAGINASE IN THE COURSE OF LIVER CANCER PRODUCTION OF RATS FED ON AZO DYE

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As far as we know, Mizuhara[4] has first introduced asparaginase into the chemical study of malignant tumors. He determined the activity of liver asparaginase in rabbits, in which transplantable sarcoma of Kato strain has been inoculated, and found marked lowering of its activity especially in the later stage. According to his assumption, this lowering might have been caused by the failure of metabolism of the liver affected by the tumor directly or indirectly. Moreover, as he stated, cachexia caused by tumor is easily distinguishable from that due to inanition, as in the latter case the activity of liver asparaginase increased.

Recently Greenstein and his collaborators[1] studied liver asparaginase in albino rats and mice. They found that there are two sorts of asparaginase, one of which, asparaginase II, is relatively acid and heat stable than the other. This is proper to hepatic tissue and is characterized by the fact that it is activated by pyruvic acid. Its activity becomes lower the more the liver tissue suffers from the effect of malignant tumor.

In recent years we have studied some liver enzyme activities in rat fed with p-dimethylaminoazobenzene (DAB), before and during the developement of the tumor. On esterase and cathepsin we have reported in recent papers.[2] This time results of the study on asparaginase will be stated.

Special attention has been payed to the possibility of carcinogenic azo dyestuff itself having some inhibiting power on enzyme activity, as Miller and Miller[3] have reported that azo dyestuff firmly combines with liver protein as it is taken by albino rats with food.

METHODS

Experimental animals.——Albino rats of mixed strain, weighing about 100 g were divided into 9 groups and were fed as follows:

Group 1, normal rats; fed with normal diet (rice grains) for more than 2 weeks.

Group 2, soy bean oil fed rats; fed with mixed diet of 1 kg rice and 20 g soy bean oil for more than 2 weeks.

Group 3, liver powder fed rats; fed with mixed diet of 900 g rice, 100 g beef

liver powder and 20 g soy bean oil for more than 2 weeks. The first three groups were used as control rats.

Group 4, DAB rats (long period of feeding); fed with DAB diet consisting of 1 kg rice and 0.6 g DAB in 20 g soy bean oil, for 9–20 weeks continuously until the day of experiment.

Group 5, DAB rats (once interrupted after long period of feeding), fed with DAB diet for 9–20 weeks, after which DAB feeding discontinued and fed on a normal diet for additional 2–3 weeks.

Group 6, DAB rats (short period of feeding); fed with DAB diet as mentioned above for 1–4 weeks continuously until the day of experiment.

Group 7, DAB liver powder rats (short period of feeding); fed with the mixed diet consisting of 900 g rice, 100 g beef liver powder and 0.6 g DAB in 20 g soy bean oil, for 1–4 weeks continuously until the day of experiment.

Group 8, OAT rats (short period of feeding); fed with the 0.06 per cent o-aminoazotoluene (OAT) diet, which had similar constituent as DAB diet, for 1–4 weeks continuously until the day of experiment.

Group 9, AB rats (short period of feeding); fed with the 0.06 per cent p-aminoazobenzene (AB) diet for 1–4 weeks continuously until the time of experiment.

Green vegetable was given occasionally to rats of all groups and dried fish powder to all except Groups 3 and 7.

Enzyme preparation.——The hepatic tissues were excised from the animal immediately after decapitation and then made clean from blood by blotting paper, weighed and triturated. Then glycerol was added to the tissue paste up to 10 times its volume (cc) calculated from the weight (g) and was thoroughly shaken. The glycerol extract was used as enzyme solution within 1–2 hours, without introducing any further procedure.

Estimation of asparaginase activity.——First of all the solution mixtures were prepared in a large (200×25 mm) thick wall pyrex test-tubes as follows: To 2 cc of the enzyme solution (corresponding to 0.2 g of the original fresh tissue), 4 cc of 0.1 M asparagine solution (substrate), which had been previously adjusted to pH 7.5 with 0.1 N NaOH, and 4 cc of 0.1 M buffer solution pH 8 (phosphate buffer) were added. On other occasions pH was adjusted to 9, 10, or 11 using glycocoll-NaOH buffer. The mixtures, overlayed with toluene, were incubated at 38°C for 24 hours, stoppered tightly. The mixture without substrate and one solely with substrate and buffer solution were used as blank tests.

During the incubation pH of the mixtures deviated somewhat from each starting pH towards acid side till the end of incubation except in the case of pH 8, which remained unchanged.

The determination of decomposed asparagine was carried out after Folin's ammonia determination method. Namely, the liberation of ammonia was accomp-

lished by addition of 5 cc saturated K_2CO_3 solution and the ammonia was transfered into 10 cc of 0.02 N H_2SO_4 by aeration for 2 hours.

The excess of acid was titrated by means of 0.02 N NaOH using methylred as indicator.

The extent of activity was presented as follows: the number of cubic centimeters of neutralized acid from which cubic centimeters of blank tests were subtracted, was multiplied by the factor 1.4 to obtain the amount of nitrogen in mg of ammonia, which has been split off by asparaginase contained in 1 g of fresh hepatic tissue at 38°C for 24 hours, under coexistence of surplus amount of asparagine.

Results

Activity of liver asparaginase of normal rats (Group 1) was generally high in an average, though there was some fluctuations. In rats fed rice containing 2 per cent of soy bean oil (Group 2) it was nearly the same as that of normal rats. On the contrary, in the rats fed with 10 per cent beef liver powder diet (Group 3) activity of liver asparaginase was increased within a few weeks so high as twice as that of normal rats (Table 1, Chart 1). In the rats which were placed on the 0.06 per cent DAB diet for 9–20 weeks (Group 4) their liver asparaginase showed very slight activity, namely, about 1/5—1/9 of normal value. In these cases the pathological changes were already recognized in livers, namely, macroscopically normal liver, liver with uneven surface, cirrhotic liver or hepatoma. Nevertheless the relation between activities of this enzyme and the extent of pathological

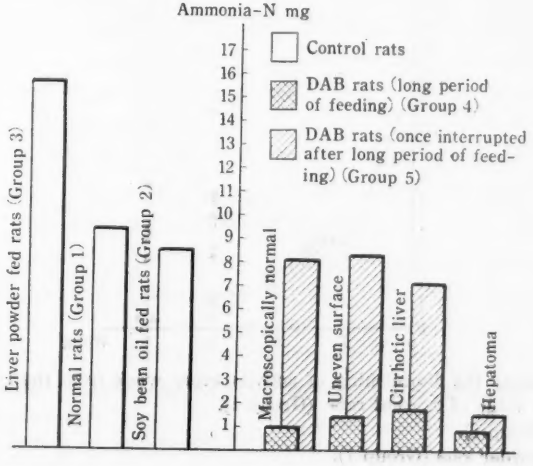


Chart 1
Comparing the activities of liver asparaginase at pH 9. of rats fed diet freed from DAB and DAB diet.

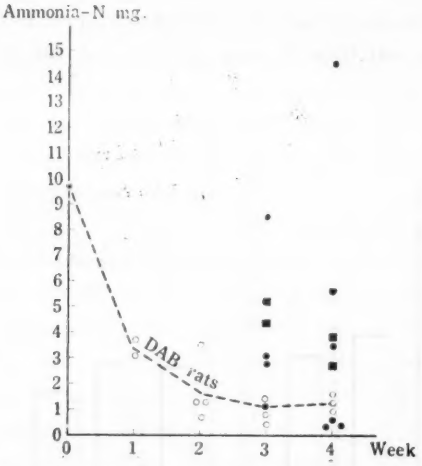
Table 1.

Group	Control rats	No. of exp.	pH			
			8	9	10	11
1	Normal rats	12	9.27 15.9 - 4.06	9.45 17.3 - 3.36	10.44 19.0 - 5.18	10.06 17.2 - 5.46
2	Soy bean oil fed rats	4	8.12 10.5 - 6.44	8.35 11.3 - 6.86	8.87 12.1 - 7.56	8.76 11.4 - 7.42
3	Liver powder fed rats	14	14.3 26.6 - 10.0	15.6 26.7 - 9.24	15.8 23.9 - 11.2	14.7 23.9 - 10.5

Numbers represent ammonia nitrogen in mg, which was digested from asparagine by enzyme solution in one gramme of fresh tissue at 38°C for 24 hours and were used in Table 2 and Charts 1—3.

changes were not decidedly clear (Table 2, Chart 1).

In rats which were fed on DAB diet for 9—20 weeks similary to the above rats, but were interrupted and placed then on normal diet for 2—3 weeks (Group 5) liver asparaginase was nearly as active as in normal liver, notwithstanding their pathological chainges. In cases of hepatoma asparaginase activity showed always the lowest value, without relating to whether DAB feeding was continued or discontinued (Table 2, Cnart 1).



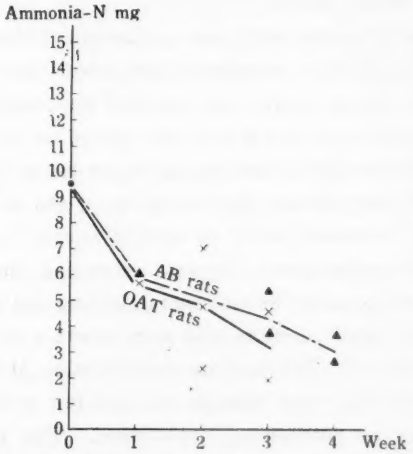
The curve represents the diminishing of activity every week from the time of DAB fed.

⊙ The average value of normal rats (Group 1).
○ DAB rats (Group 6).
● DAB-liver powder rats (Group 7).
■ The rats (Group 7) fed thoroughly mixed diet.

Chart 2
Activity of liver asparaginase at pH 9.

Table 2

DAB rats	Liver findings	No. of exp.	pH			
			8	9	10	11
Group 4 (long period of feeding)	Macroscopically normal	3	0.99 1.17 - 0.89	0.91 1.0 - 0.86	0.91 1.12 - 0.78	0.85 1.0 - 0.78
	Uneven surface	2	1.27 1.34 - 1.2	1.27 1.34 - 1.2	1.44 1.6 - 1.28	1.41 1.6 - 1.23
	Cirrhotic liver	2	1.75 2.24 - 1.27	1.75 2.24 - 1.27	2.15 3.08 - 1.22	2.26 3.36 - 1.16
	Hepatoma	1	0.7	0.84	0.98	1.12
Group 5 (once interrupted after long period of feeding)	Macroscopically normal	5	8.11 10.9 - 3.64	8.05 10.3 - 3.78	8.86 11.0 - 4.62	8.23 9.8 - 4.34
	Uneven surface	5	8.96 12.1 - 5.74	8.34 10.7 - 6.02	8.68 11.2 - 6.3	6.81 11.0 - 6.44
	Cirrhotic liver	22	7.31 11.3 - 2.38	7.05 9.8 - 2.52	8.12 10.6 - 3.78	7.95 10.9 - 3.64
	Hepatoma	5	1.28 1.96 - 0.84	1.34 1.82 - 0.7	1.66 2.38 - 0.7	1.68 2.66 - 0.7



The curves represent the diminishing of activity every week from the time of OAT and AB fed respectively.

⊙ The average value of narmal rats (Group 1).

× OAT rats (Group 8).

▲ AB rats (Group 9).

Chart 3

Activity of liver asparaginase at pH 9.

In the early period of DAB feeding (1—4 weeks) the rats (Group 6) indicated the lowering of the enzyme activity every week. At the end of the first week the activity was 1/3 of normal value and after 3—4 weeks reached the lowest value (Chart 2).

When the rats were fed with the diet containing 0.06 per cent DAB and 10 per cent beef liver powder (Group 7) the activity was very variable and some of them were extremely high even after 3—4 weeks of feeding (Chart 2). From four rats of Group 7, fed with all ingredients diet similar results were obtained (Chart 2). With another azo dye, 0.06 per cent OAT (Group 8) or 0.06 per cent AB (Group 9), the feeding experiments were carried out for 1—4 weeks, and their diminishing actions on the enzyme activity were always less than that of DAB (Chart 2 and 3).

Discussion

In the early period of DAB feeding, in which practically no pathological changes has been developed in liver, the activity of asparaginase was markedly lowered. We thought it might have been caused merely by intoxication of DAB. DAB is bound firmly to liver protein, as Miller et al. said, and it may be bound also to the protein portion of asparaginase, inhibiting its action as enzyme toxin. There can be seen a fair coincidence between the lapse of time, when the amount of bound dye in the liver reaches the maximum, and that when the asparaginase activity comes to the lowest value.

During DAB feeding was continued and pathological changes taking place in the liver, asparaginase activity maintained the lowest value. Nevertheless the activity of asparaginase by no means was lost until the change becomes cancerous. When the rats were freed from DAB diet and placed on the normal diet for the lapse of time sufficient for the hepatic tissue to get rid of DAB intoxication, the enzyme activity of the macroscopically normal liver, the liver of uneven surface, and the cirrhotic liver recovered nearly to that of normal liver. Then the livers, though yet with some pathological changes, recovered their original enzymatic activities, being affected no more by the contaminating DAB.

In the hepatoma the lowest activity was seen whether DAB feeding was kept continuously or discontinued. Taking into consideration Miller's report, that the hepatoma has no bound dye even though rats are fed with the dye, the above mentioned fact arouses an interesting implication. The type of liver enzyme changes suddenly associated with the cancerisation of the tissue. Lowering of asparaginase activity appears to be one of those influences.

In rats fed with liver powder the activity of liver asparaginase showed extremely high value and in those fed with liver powder and DAB at the same time liver powder appeared to have an antagonistic action against DAB, which depressed

the asparaginase activity. Nakahara and his colleagues[5] previously reported that the beef liver powder has a marked inhibiting power against liver cancer produced by DAB. Consequently our findings may afford some biochemical support to their remarkable finding. If beef liver diet has an antagonistic power against DAB stress, liver function recovers to the begining state each time and this may well result in the retarded occurrence of hepatoma.

In the early stage of azo dye feeding, DAB (strong carcinogen) OAT (less strong carcinogen) and AB (non-carcinogenic) were compared regarding their diminishing power on asparaginase activity and DAB was found the highest in its power. With this experiment relative carcinogenic potencies of azo dyes might be explained to some extent.

Summary

1) In albino rats fed on liver-carcinogenic diet containing DAB trend of the liver asparaginase activity was studied from early to later stages of experimental days, till developement of hepatoma.

2) A few weeks after the experiment has been started, marked lowering of the liver asparaginase activity compared to that of normal was observed and this phenomenon was seen as long as DAB was added to the diet. Also in the later period, when the pathological changes of the liver occurred, the activity remained still very low.

3) A group of rats was fed with DAB diet for a long period, thereafter addition of DAB was discontinued and was fed on normal diet for a while. Their liver asparaginase activity recovered nearly to normal, notwithstanding some pathological changes. In cases of hepatoma, on the contrary, the enzyme activity has never recovered.

4) The asparaginase activity of hepatoma showed always the lowest value not only in the experiments where DAB feeding was discontinued, but also in those where DAB was given continuously.

5) Activity of liver asparaginase in rats fed with normal diet added with beef liver powder for a few weeks showed extremely higher value than that of normal rats. In cases of the rats fed DAB and liver powder simultaneously, the activity was variable, though some of them were very high.

6) In experiments with other azo dyes, OAT and AB were used besides DAB. In the early period of feeding the inhibiting actions of these azo dyes on the liver asparaginase activity were generally lower than that of DAB, which was most active among them.

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要　旨

アゾ色素投與による白鼠肝癌生成過程における肝アスパラギナーゼについて

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発癌アゾ色素バターエロー（DAB）投与の白鼠肝アスパラギナーゼ（Asp.）の活性度を初期より末期の肝癌生成迄追跡測定した，初期に強度に低下し，3～4 週で最低値に近い値を示した。しかして長期 DAB 投与により肝に病変が表われても同様であった。もし長期 DAB 投与の後，投与を中絶し普通食で飼育した白鼠肝 Asp. の活性度は肝癌以外の病変肝は正常値に近い値を示した。これは病変肝の DAB の影響のない真の Asp. 作用と考える。実験初期よりその低下をみたのは DAB が酵素毒として働いたものと考える。肝癌は中絶実験でも最低値であるのは癌化と同時に一般に酵素の型が変化し，その現われの一つとして Asp. の活性度の低下となったものと思う。

牛肝末を普通食に添加飼育した場合の肝 Asp. の活性度は正常値より極めて高い，DAB と同時に牛肝末を与えた結果はさまざまで 3～4 週後にもなお極めて高い値を示したものもあった。牛肝末の制癌物質であることの一つの生化学的説明に役立つと考える。

他のアゾ色素，o-アミノアゾトルオール，p-アミノアゾベンツオール投与実験の初期において肝 Asp. 活性度を低下させる作用の強さは DAB（強発癌性）に劣ることを知った。

（文部省科学研究費による）

[GANN, Vol. 43; Dec., 1952]

CATALASE STUDIES ON RATS DURING p-DIMETHYLAMINO-AZOBENZENE CARCINOGENESIS*

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It is reported by several workers[1-7] that the activity of liver catalase is definitely reduced in the process of liver cancer production by butter yellow (p-Dimethylaminoazobenzene) feeding. Above all, Nakahara and Fukuoka[3,5] demonstrated that the reduction in the liver catalase activity may have a significant role in the production of liver cancer by the azo-dye. Greenstein[2] and Applemen and coworkers[7] reported that with the progressive growth of the transplanted tumor there is a marked decrease in the liver catalase activity, and that kidney and erythrocyte catalase are affected but slightly, if at all, by the presence of a tumor.

In this paper, we propose to report our experiments showing that the decrease of the catalase activity of the liver of rats was less marked when the animals were placed on a normal diet after dye feeding than when were fed on dye diet continuously. In these experiments the kidney catalase activity during the course of liver cancer production by butter yellow feeding was also studied.

METHODS

The quantitative determination of the catalase activity was made by the gas-volumetric method, using a Battelli-Stern apparatus, as described by previous investigators.[3,5] The duration of the test was 5 minutes at room temperature. The value of oxygen obtained by the action of catalase was calculated into the standard condition (0°C, 1 atm. press).

In expressing the nature of the liver findings, four grades of the pathological changes leading to the production of liver cancer were indicated as macroscopically normal, uneven surface with slight connective tissue proliferation, cirrhotic liver and liver cancer. The identification of these liver findings has been described in detail in the publication cited.[8]

EXPERIMENTAL RESULTS

Preliminary experiment: To determine the optimum acidity for the catalase activity, firstly, the catalase of the liver and kidney of the normal male rats was

* Aided by a Scientific Research Encouragement Grant from the Ministry of Education

estimated at various ranges of hydrogen-ion-concentration. The acidity of the buffer solution ranged from pH 3 to pH 11. Solutions of pH 3 and 4 were made of the glycocoll-HCl, and solutions ranged from pHs 8.5 to 11 were made of the glycocoll-NaOH mixture. Solutions of pH ranges between pH 5 and pH 8 were the phosphate mixtures. The acidity of the solution was measured by pH electrometer.

Table 1.

Catalase Activity of the Liver and Kidney of normal Rats in various Acidities (pH 3-11).

(Figures given as averages and ranges)

pH in unit	3	4	5	6	7	8	8.5	9	10	11
Liver	0.2 0.0-0.4	1.0 0.1-1.7	1.5 0.6-2.4	2.2 1.1-3.3	4.5 2.9-6.1	5.4 3.0-7.6	5.8 4.1-7.4	5.5 3.6-7.4	1.1 0.2-1.9	0.5 0.0-0.7
Kidney	0.4 0.0-0.8	0.6 0.4-0.8	0.7 0.5-0.9	2.0 1.1-2.7	2.6 1.3-3.9	3.5 2.0-4.9	3.8 2.5-5.1	3.5 2.1-5.0	0.9 0.2-1.2	0.4 0.3-0.5

The result is shown in Table 1. It is demonstrated in the table that both liver and kidney catalase showed the maximum activity at pH 8.5. In all following experiments the catalase was estimated at pH 8.5.

Experiment 2: At various periods after being fed on diet containing 0.06 per cent of butter yellow for 12 or 14 weeks, the rat was killed by exsanguination and the catalase activity of the liver and kidney was determined according to the method described above. As may be apparent from Table 2, which shows the

Table 2.

Catalase Activity of the Liver and Kidney of Rats fed Butter Yellow continuously (pH 8.5)

(Figures given as averages and ranges)

Liver Findings	No. of Rats	Liver Catalase	Kidney Catalase
Control	10	5.8 4.1-7.4	3.8 2.5-5.1
Macroscopically normal	7	5.2 3.8-7.1	4.4 3.1-5.4
Uneven surface	7	4.3 2.0-6.4	4.4 2.8-5.5
Crrhosis	10	2.7 1.4-4.2	3.7 2.7-4.6
Liver cancer	5	0.8 0.1-1.3	4.7 3.7-5.5

result of this experiment, there was a demonstrable reduction in the catalase activity of the liver. These experimental data of the liver catalase are in a close agreement with the results obtained by the previous investigators.[3,5]

In the case of kidney catalase activity, there were an initial elevation following a slight lowering and a striking elevation eventually. These elevation of kidney catalase activity in a tumor-bearing rat is not noticed by previous investigators.[2]

Experiment 3: In this experiment, the animals were maintained on the diet with the azo-dye for 12 or 14 weeks. After the completion of dye feeding period, the animals were placed on a normal rice diet without the carcinogen for an additional 2 or 3 weeks to permit the dye in the rat tissues to disappear.

Animals were sacrificed by exsanguination and the catalase activity of liver and kidney was estimated just the same as in Experiment 2. Table 3 represents the results of this experiment. It may be readily seen that the liver catalase shows no marked reduction in the activity, and at an early period the activity may even be slightly higher than normal. In the cirrhotic liver, the catalase activity shows slight decrease. But when the liver tissue turned cancerous, the catalase was reduced in its activity definitely. It is reasonable that the catalase activity shows a slight reduction in the cirrhotic liver in which active liver cells may be reduced in number owing to the proliferation of the connective tissues when compared to the normal.

In the same table, it will be seen that the kidney catalase is affected but slightly in the course of liver cancer production.

Chart 1 graphically shows the average changes in the activity of liver catalase

Table 3
Catalase Activity of the Liver and Kidney of Rats fed Butter Yellow for 12 or 14 weeks and then placed on Normal Diet for 2 or 3 weeks (pH 8.5).
(Figures given as averages and ranges)

Liver Findings	No. of Rats	Liver Catalase	Kidney Catalase
Control	10	5.8 4.1 - 7.4	3.8 2.5 - 5.1
Macroscopically normal	6	6.0 5.4 - 6.9	4.5 4.2 - 4.8
Uneven surface	10	6.2 4.5 - 7.5	4.4 3.5 - 5.0
Cirrhosis	20	4.7 3.9 - 5.4	3.6 2.4 - 4.5
Liver cancer	7	0.9 0.3 - 1.7	4.2 2.3 - 6.3

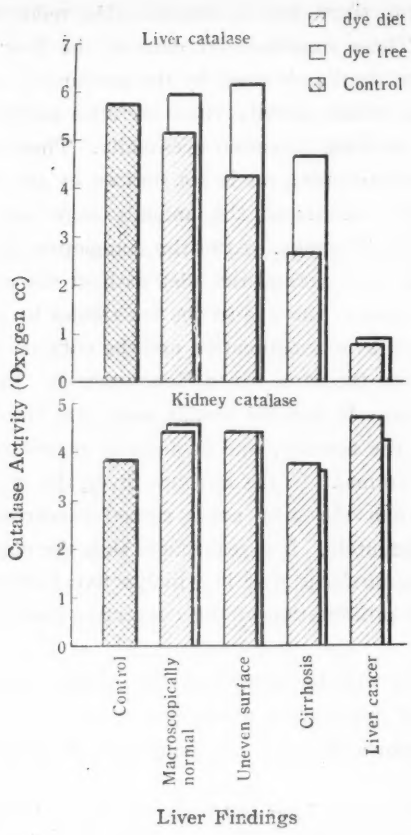

Chart 1

Average Changes in the Catalase Activity of the Liver in the Course of Liver Cancer Production (pH 8.5)

of rats fed dye diet continuously, as contrasted to that of rats fed dye-free diet for 2 or 3 weeks after the dye feeding period during the course of liver cancer production.

Summary and Conclusion

It has been demonstrated that butter yellow feeding definitely reduces the liver catalase activity in the course of liver cancer production. However, a further investigation showed that this reduction in the liver catalase activity becomes less marked when the animals are placed on normal diet after a difinite period of the dye feeding. Miller and his associates[9,10] have reported that the tissues of rats fed butter yellow contain more or less bound dye which decreases rapidly when the feeding of dye is stopped and there is no bound dye in the liver cancer

and kidney. The difference in the liver catalase activity between dye fed and non-dye fed rats may be related intimately to the concentration of the dye in the tissues.

It is noteworthy that in the liver cancer, however, no difference in the catalase activity is to be seen between the rat fed diet with and without butter yellow.

Also it was demonstrated that the kidney catalase tended to be unaffected or slightly higher than that of the corresponding controls during the butter yellow carcinogenesis.

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要　旨

実験的肝癌成生過程におけるカタラーゼの研究

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木下法にしたがい，Butter Yellow 白米食で白鼠を飼育すること 14 週後，これらの動物を2群に分けた。1 群はさらに Butter Yellow 白米食を与えつづけ，他の 1 群は正常食に切替え，2 週乃至 3 週を経て実験に供した。

実験に際し，白鼠を出血死せしめ，その肝の病変にしたがって肉眼的正常，表面不平滑，肝硬変並に肝癌の 4 段階に分け，その各々の肝並に腎カタラーゼ作用を Battelli-Stern 装置により測定比較検討した。

予備実験に際し，カタラーゼ作用の至適酸度が pH 8.5 であることを先ず確め第 1 表に示した。

次に第 1 群の白鼠の肝並に腎カタラーゼ作用を第 2 表に示した。表に明らかなように肝の病変が進行するにつれて，動物の肝カタラーゼ作用は漸減し，肝癌では 0 に近いかあるいは痕跡的存在を示すに過ぎなかつた。これらの成績は従来の諸研究者の報告に完全に一致している。他方腎カタラーゼ作用は肝カタラーゼの減少につれ逆に漸増している点が注目に値する。

第 2 群即ち Butter Yellow 白米食後正常食を与えた動物の肝並に肝カタラーゼ作用は，第 3 表にみる如く上述の結果とは異った傾向を示した。即ち肝の病変の初期では肝カタラーゼ作用はむしろ増加している。硬変を呈する肝のカタラーゼ作用も正常肝の 80% 程度を示している。しかし肝癌では第1群の白鼠の場合と同様に痕跡的であった。腎カタラーゼは第1群の場合ほど顕著ではないが病変の進行につれて多くの場合正常値よりうわまわっている。

さて上述の諸結果は第 1 図に図示して参考とした。第1群並に第 2 群の動物の肝カタラーゼの差異は何に由来するのであろうか。著者等は必ずしも結論を急ぐものではないが，肝組織に含まれる Butter Yellow の有無は大いに関係あるのではなかろうか，殊に Miller 等の報告による Butter Yellow 飼与動物の肝組織に存在する蛋白結合色素の意義は見のがすわけにはいかない。

（文部省科学研究費による）

[GANN, Vol. 43; Dec., 1952]

STUDIES ON THE IRON CONTENT OF RAT LIVER DURING p-DIMETHYLAMINOAZOBENZENE CARCINOGENESIS*

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Recently, attempts to follow the behavior of the liver catalase in the cancerous animals were reported by several workers.[1-3] As the physiological significance of this enzyme, which represents an iron-porphyrin system, cannot be over looked in the liver cancer production, the total iron content in rat liver before and during development of a tumor has been investigated in the following experiments.

MATERIAL and METHODS

A group of normal male albino rats was maintained on the usual butter yellow (p-Dimethylaminoazobenzene) diet for 12 or 14 weeks. After the dye-feeding period animals were continued on the basal diet free from dye for 2 or 3 weeks. Then the rat was sacrificed by exsanguination at random and the lobes of the liver were cut, weighed and dried in the desiccator to the constant weight. From the total wet liver weight and the water loss of the duplicate samples, the total dry weight was culculated.

In expressing the nature of liver findings, four grades of the pathological changes leading to the production of liver cancer were indicated as macroscopically normal, uneven surface with slight connective tissue proliferation, cirrhotic liver and liver cancer. The identification of these liver findings has been described in the previous publication.[4]

For the determination of iron, the method of Kennedy[5] was used. 100 mg of dried sample was transferred into a large pyrex tube and was oxidized into a colorless solution by heating with the addition of 3 cc of concentrated H_2SO_4 and a few drops of perhydrol. The whole was cooled, a drop of nitric acid was added, and was diluted to 50 cc. Then 10 cc portions of solutions was measured into 50 cc glass stopped cylinders, and 10 cc of amyl alchohol and 5 cc of 20 per cent solution of potassium thiocyanate were added. Then the mixture was shaken throughly, the colored layer of amyl alcohol was removed with a pipette and the intensity of the color was estimated with the aid of Hitachi electro-colorimeter. And the degree of the concentration of sample solution was compared to the standard solution of iron. Blank test was always conducted in each series of the experiment.

* Aided by a Scientific Research Encouragement Grant from the Ministry of Education.

Table 1. Data on Individual Rats

Liver Findings	Normal (Control)		Macroscopically Normal		Uneven Surface		Cirrhosis only		Cirrhotic Lesions in the liver with Cancer nodules		Liver Cancer	
No. of Rats	15		12		11		9		14		10	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Iron content (mg/dl)	0.0196	0.0059	0.0461	0.0152	0.0297	0.0100	0.0291	0.0088	0.0251	0.0059	0.0126	0.0029
	0.0187	0.0057	0.0460	0.0121	0.0297	0.0099	0.0211	0.0063	0.0196	0.0059	0.0122	0.0027
	0.0187	0.0039	0.0447	0.0090	0.0295	0.0092	0.0173	0.0052	0.0191	0.0072	0.0102	0.0024
	0.0182	0.0057	0.0421	0.0105	0.0259	0.0078	0.0164	0.0049	0.0186	0.0055	0.0093	0.0019
	0.0161	0.0051	0.0321	0.0096	0.0248	0.0074	0.0143	0.0043	0.0182	0.0056	0.0089	0.0017
	0.0155	0.0052	0.0313	0.0097	0.0189	0.0062	0.0106	0.0033	0.0181	0.0052	0.0061	0.0016
	0.0141	0.0046	0.0286	0.0090	0.0189	0.0059	0.0091	0.0028	0.0158	0.0048	0.0044	0.0008
	0.0127	0.0042	0.0274	0.0082	0.0184	0.0056	0.0081	0.0022	0.0153	0.0048	0.0041	0.0010
	0.0107	0.0035	0.0249	0.0075	0.0179	0.0055	0.0036	0.0010	0.0133	0.0040	0.0036	0.0007
	0.0105	0.0033	0.0174	0.0057	0.0168	0.0055			0.0116	0.0032	0.0032	0.0007
	0.0095	0.0028	0.0106	0.0029	0.0154	0.0048			0.0116	0.0021		
	0.0088	0.0025	0.0092	0.0032					0.0109	0.0034		
	0.0072	0.0024							0.0096	0.0030		
	0.0072	0.0023							0.0066	0.0020		
	0.0062	0.0020										
Average	0.0129	0.0039	0.0300	0.0085	0.0223	0.0071	0.0144	0.0043	0.0152	0.0045	0.0075	0.0016

Results

The data for each rat are recorded in Table 1, in which the values of iron content of the dried liver are listed in descending order of mg per cent. The table also includes the iron content of the liver in wet which was calculated from the water content. And the results are shown in Chart 1 graphically. It is readily seen that the iron content of the liver was more than twice the normal value when the liver appeared macroscopically normal, but there was a gradual decrease in the iron content, which reached quite a marked degree in the liver cancer.

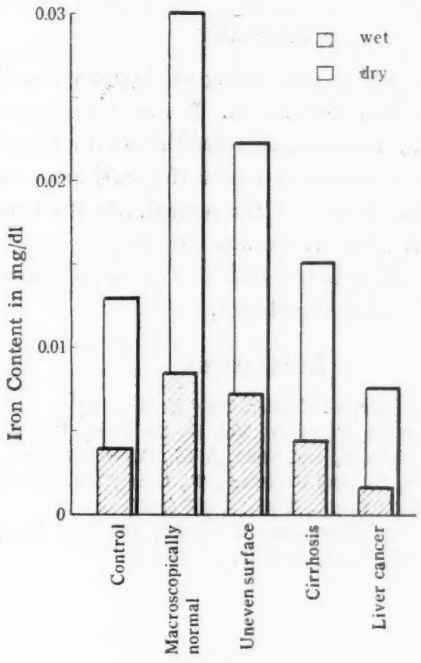

Chart 1.

Average Changes in the Iron Content of the Liver of Rats in the Course of Liver Cancer Production

And no difference in the iron content was detected between the liver tissue which shows a typical cirrhosis only and the cirrhotic regions in the liver in which more or less solid whitish cancerous nodules were seen. These values of the iron content in the control liver and the cancer tissue obtained in this study are in a close agreement with results obtained by the previous investigators[6] who studied the iron content of the transplantable hepatoma caused by o-aminoazotoluol.

The iron content of the liver in wet showed the similar tendency to that of the dried liver as shown in the table.

According to the recent discovery[7] in this laboratory, the liver catalase shows only slight decrease in its activity in the cirrhotic liver, but definite reduction in the hepatoma when compared with the normal liver. There was no remarkable elevation in the liver catalase activity in the early hepatic change in which the iron content showed a peak as described above, Thus the total content of the liver iron may not be proportional to the catalase activity in the course of liver cancer production. It seems probable that the major source of liver iron is the destruction of red blood cells.

Summary

The iron content of the liver of rats during the butter yellow (p-dimethylamino-azobenzene) carcinogenesis was determined. It was demonstrated that there was an initial elevation, which, however, decreased gradually proportional to hepatic changes. The peak of the iron content was at the early stage of the liver changes and the content was more than twice the normal. In the liver cancer, the iron content showed the lowest value which was only 58 per cent of normal.

At the end of this paper it is pleasure to express our appreciation to Dr. S. Kishi for his kind advice on the experiment.

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要　旨

肝癌生成過程における白鼠の肝鉄量の研究

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白鼠に Butter Yellow 白米食を与え 14 週を経て後，正常白米食に替え更に 2 週乃至 3 週飼養した。これらの動物を出血死せしめ，肝病変を肉眼的正常，表面不平滑，肝硬変乃至肝癌の 4 段階に分ち，その各々をデシケーター中で減圧乾燥秤量した。乾燥材料 100 mg 宛を採り，H_2SO_4 酸化後，KSCN で呈色せしめ光電比色計を用いて，その全鉄量を比色定量した (Kennedy 法)。

実験結果は第 1 表に示し，第 1 図に図示した。即ち肝癌生成過程における肝鉄量の消長は乾燥，新鮮両材料共に同じような傾向を示している。即ち肉眼的正常肝の全鉄量は全例中最高値を占め，正常肝の 2 倍以上に達した。ついで表面不平滑肝並びに肝硬変の場合はこれより漸減し，肝癌の際は正常肝の半減値にすぎなかつた。

（文部省科学研究費による）

[GANN, Vol. 43; Dec., 1952]

THE PRODUCTION OF GASTRIC LESIONS IN THE RAT BY ACETIC ACID FEEDING.*
(WITH PLATE XIV)

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The development of gastric lesions in the rats receiving diets containing acetic acid was discovered in this laboratory. And the results are reported in the following lines.

Experimental Results

Experimental 1: 20 albino rats were maintained on rice, to which glacial acetic acid was mixed evenly. The amount of acetic acid added was 50 cc per 1 kg of rice through out the experiment. Usually, a small amount of cod liver oil was introduced along with acetic acid on the assumption that the dissipation of acetic acid from the surface of rice grains may be somewhat hindered by so doing. No attempt was made to estimate the actual amount of acetic acid ingested by the rats as the volatility of acetic acid rendered any such attempt practically impossible. In view of the deficient nature of the diet, green vegetables were given to the rats from time to time.

Under the above experimental conditions, 10 rats were killed by carotid bleeding on the 30th day, and the stomachs were removed and placed in a fixative. After slight fixation, the stomachs were cut along the greater curvature. The tissues were embeded in paraffin and sectioned and stained routinely with hematoxylin and eosin.

In 3 of the 10 cases, a few umbilicate lesions were evident, and in 3 others slight thickening of the wall was seen in the forestomach, and the remaining 4 cases showed no gastric change. Other 5 rats were sacrificed on the 166th day, followed by autopsy. In 4 of the 5 cases, the umbilicate lesions or papillomatous growths were demonstrated in the forestomach.

All other rats were killed on the 200th day and the majority of them showed more or less definite gastric lesions as may be seen from the following table (Table 1):—

* Aided by a Scientific Research Encouragement Grant from the Ministry of Education.

Table 1

Rat No.	Sex	Period of Feeding (Days)	Body Weight (g)			Liver Weight (g)	Spleen Weight (g)	Gastric Lesions
			Initial	Maximum	Final			
1	♂	30	70	95	95	4.8	0.5	±
2	♂	30	65	85	80	4.2	0.5	−
3	♂	30	68	82	82	4.4	0.8	−
4	♂	30	60	65	65	3.8	0.4	−
5	♂	30	72	90	90	4.5	0.6	±
6	♂	30	65	80	80	3.5	0.4	±
7	♂	30	75	105	100	5.2	0.6	+
8	♂	30	68	85	82	4.0	0.4	+
9	♂	30	65	88	88	3.7	0.6	+
10	♂	30	65	85	85	5.0	0.6	−
11	♀	166	117	175	150	7.8	0.5	+
12	♂	166	87	145	120	8.0	0.4	−
13	♂	166	152	202	162	8.2	1.0	+
14	♀	166	95	167	152	8.3	0.6	+
15	♀	166	97	159	110	4.0	0.6	+
16	♀	200	130	205	200	8.8	0.8	+
17	♀	200	117	195	170	8.2	0.6	+
18	♀	200	122	203	203	8.8	0.8	+
19	♀	200	152	197	168	8.2	1.0	+
20	♀	200	95	170	170	7.6	0.6	+

In expressing the nature of gastric lesions the use was made of plus (+), plus-minus (±) and minus (−) signs indicating, respectively, umbilicate lesions, acanthosis or hyperkeratosis and macroscopically normal. In all cases, these diagnoses were comfirmed by microscopical examination.

Three examples of the gastric lesions are illustrated in Fig. 1.

In the above experiments, it is demonstrated that the gastric changes in the rats will be produced within 30 days after the beginning of the experiment. No remarkable change was found in other organs.

Experiment 2: In another experiment, in which the amount of acetic acid was greatly reduced in the beginning of the experiment, and the gastric changes were less remarked than in the above experiment. In this group, the amount of acetic acid added to rice varied. It was started with a small amount, 10 cc per 1 kg of rice, and was gradually raised up to 50 cc per 1 kg. Duration was 10 cc per 1 kg of rice for 18 days, 20 cc for 157 days and 50 cc for the rest of the experimental period. On the 200 th day after the commencement of the experiment one rat died with umbilicate lesions in the forestomach. On the 250 th day, half of the remaining rats were killed and the rest of animals were sacrificied on the 325 th day, performing autopsy respectively. The results of the above experiment are

shown in the following table (Table 2).

Table 2.

Rat No.	Sex	Period of Feeding	Body Weight (g)			Liver Weight (g)	Spleen Weight (g)	Gastric Lesions
			Initial	Maximum	Final			
21	♂	200	102	102	65	6.2	0.4	+
22	♂	250	100	155	130	7.2	0.6	±
23	♂	250	142	167	130	7.1	0.5	+
24	♂	325	130	190	190	9.0	0.9	±
25	♂	325	140	170	137	7.6	0.8	−

In this table, 2 of the 5 cases showed umbilicate lesions scattered in the forestomachs and 2 others demonstrated slight hyperkeratosis and the remaing 1 case was macroscopically normal. In the above results, it is demonstrated that time required for the production of gastric changes is greatly prolonged when the amount of acetic acid was reduced.

Pathological Note

Grossly, in the forestomachs, the walls were opague, gray or white, indurated and rigid. The serosa, however, was glistening and smooth except at the base of gastric lesions where the wall was often retracted. The limiting ridge was proniment and often beaded. The gastric lesions varied from small, pin-point to large excrescenses, and usually a small bloody red spot was detected at the top of the localized lesions.

Microscopically, the lesions were composed of proliferations of hyperplastic epithelium thrown into convoluted folds, and these proliferations were covered with keratin. A few rats in both experiments showed areas of gastritis and occasionally extensions of portions of the glandular mucosa into the submucosa.

Occasionally, spurs of hyperplastic epithelium extended downward from the papilomatous growths and penetrated the muscularis mucosa. Some of these were found deeply placed in the submucosa. In these areas mitotic figures were numerous, indicating a rapid growth of this lesion. Very often umbilicate lesions presented a defect in the keratin above it. This defect was regularly associate with an inflamatory focus in the epithelium and the underlying connective tissue. The basal layer was thickened.

The lamina propria and submucosa regularly showed an inflammatory reaction. The inflammatory cells consisted of neurotrophilic and eosinophilic leukocytes, lymphocytes, plasma cells and mononuclear leukocytes.

Discussion and Summary

Numerous investigators attempted to produce gastric lesions in the rat or mouse

with various diets or chemicals, as presented in a comprehensive review by Sugiura.[1]

The criteria adopted in this paper for the gastric lesions of the forestomach were those of the rats which received tributyrin diet. In this laboratory, more than one hundred rats were fed for 500 days with the rice diet containing 5-10 per cent of butyric acid with a small amount of cod liver oil, supplemented with green vegetables just same as the above experiment. The results were almost similar to those obtained by Salmon and Copeland.[2,3] In the experiment, the amount of the butyric acid was reduced to the level of 5-10 per cant, less than half of the amount cited by Salmon, and few rats died during the early course of the experiment. And the stomachs of all rats fed for more than 50 days showed ulcers or small discrete papillomas. As indicated by Salmon, in the rats of this laboratory numerous cysts were found penetrating the muscularis mucosa, and these cysts were lined with stratified squamous epithelium and contained keratin in a lamellated fibrillary arrangement. These results of butyric acid feeding will be reported elsewhere.

In the case of the rats fed acetic acid, no cyst or nodule containing keratin was demonstrated in the forestomach. But, except the absence of keratin cyst in the forestomach, the other gastric lesions caused by acetic acid were similar but less marked than those due to butyric acid.

It is noteworthy that the two chemicals in the low fatty acid group similarly produce the gastric lesions of rats by oral administration. A question remaines as to how propionic acid, one of the low fatty acids, act on the forestomach of the rats, and the inverstigation is now under way in this laboratory.

In conclusion the author wishes to express his sincere thanks to Dr. Sanji Kishi, Head of Biochemical Section of this School, for his valuable suggestions.

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要　旨

醋酸添加飼料による白鼠の前胃変化生成について

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白米に 5% の割合で氷醋酸を添加した飼料で，白鼠を飼養すると前胃粘膜上皮の増生及び角化，乳嘴腫様変化並びに潰瘍等が生成されることがわかった。即ち醋酸飼与 30 日以上を経た白鼠の前胃壁は一体に肥厚し透明度を減じ，処により著しく肥厚している。しかして灰白色あるいは白色で中心に紅褐色の斑点としてみえる陥落部を伴った乳嘴腫様突起が散在性にみられる。

顕微鏡的には肥厚した上皮細胞の増殖による乳嘴腫様変化で最上層の角化が著しい。そしてしばしば肥厚した上皮は筋肉層内へ侵入し異所的増殖の像を示し，その部分には多くの核分裂像をみとめることが出来る。多くの場合，乳嘴腫様変化の一部は崩壊欠如し，潰瘍を形成している。

これらの変化は実験日数 30 日で早くも出現し始めるが，200 日程度の時日を経ても乳嘴腫様変化の増殖以外に特記すべき進行性の変化はみられなかった。

従来多くの研究者によって種々の化学物質を用いて，動物の胃に腫瘍あるいは腫瘍様変化をもたらした業績が発表され，殊に杉浦博士はそれらを綜説的にまとめられた。一方 Salmon 並びに Copeland は多量の trybutyrin を飼料に添加し，白鼠の前胃に乳嘴腫を惹起せしめた。この研究室においても彼等と全く独立した見解から酪酸飼与実験を繰返した。酪酸飼与による白鼠の前胃変化は，上記の醋酸の場合によく似ている。しかし酪酸の場合に多くみられる keratin cyst は，本実験では全く出現せずに，むしろ潰瘍の生成が普通であった。

このような低級脂肪酸群中，酪酸並に醋酸の影響が著明である事から，更にプロピオン酸の作用が当然研究の対象となってくるが，実験は目下進行中である。

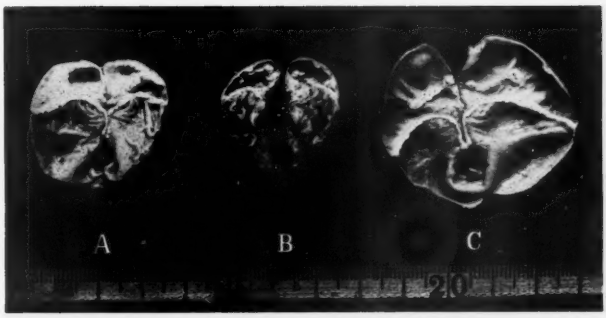

Fig. 1. Three examples of the gastric lesions. Period of acetic acid feeding 166-200 days.
A. The entire forestomach is a mass of papillomatous growths and umbilicate lesions.
B. and C. Note the craters in the forestomachs.

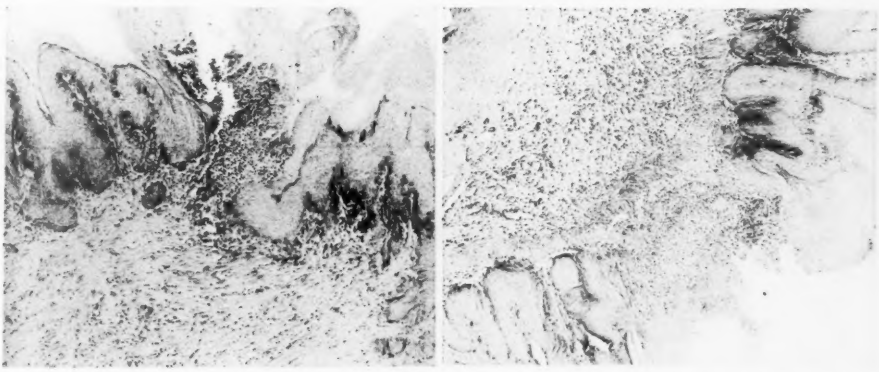

Fig. 2. Sections through umbilicate lesion to show proliferation of stratified squamous epithelium and hyperkeratosis.

[GANN, Vol. 43; Dec., 1952]

STUDIES ON THE IMMUNOLOGICAL PHENOMENA OF YOSHIDA SARCOMA

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Whether the immunization can be found in tumors or not is a problem of great importance, because its diagnosis, prophylaxis and therapy can be performed with this procedure. But many of the immunological phenomena have been proved almost only in the cases of transplanted tumors, and negative opinions were predominant mostly in original tumors, except for the special tumors caused by virus.

Since the immunological phenomenon of transplantable tumors is distinguished especially in the cases of the heterogenetic transplantation, these facts are apt to indicate that the immunity is effected only in the species-heterogeneity between tumorigenic animal tissues and transplanted animals.

It is naturally understood that tumors cannot develop in the bodies if immunity is strongly produced in them, and therefore, in this case it may be assumed that the immunity is unimportant, even if it is produced there. Therefore, there remain many things insufficiently solved in relation to the above problems by means of the usual research methods and of the ordinary solid tumors. It is necessary to get some tumor and some pure strain of animals in order to adequately study the more delicate phenomena, and to obtain finer techniques.

Our researches have developed from those of allergy and immunity in inflammation, especially in tuberculosis, to the same kind of study on the transplantable tumors, but the studies have not succeeded because tumors used were not suitable for our experiments. Fortunately an interesting ascites tumor, called Yoshida sarcoma was kindly given to us by Prof. Yoshida, and in the transplantation of it to some strains of rats we have recognized some results which should be considered as immunological phenomena. It is our main object in this paper to investigate the delicate immune phenomena of tumors from the viewpoint of allergic reaction by using this specific ascites tumor.

Behavior of Yoshida sarcoma in the successive transprantations to several strains of rats.

The lineages of animals used for the transplantation.

The tumor was transplanted in the following strains of rats:—

1) Albino rat of Yamashita strain (Y). This strain has been inbred for about 15 years from one pair of unclassified hybrid white rats in Yamashita Institute, Hokkaido University, and its behavior toward Yoshida sarcoma is pure and constant at least.

2) White rats of non classified hybrid, named Saitama- (S) and Gifu- (G) hybrid in markets. They take almost the same attitude as Y-strain toward the tumor.

3) White rats of Wistar strain (W). A thorough-bred strain of Wistar Institute, U.S.A., was further purified in the Faculty of Science, Hokkaido University (W_1) and still further in our laboratory (W_2). There are some differences in the behavior toward the tumor between these two (W_1-W_2) strains. W_2 strain was used mainly for this study.

4) Water rats (D): wild black rats, a maternal lineage of white rats, but in relation to the tumor there are differences between D_1 and D_2.

5) Roof rats (K): wild black rats different from Water rats.

6) Mice (M), rabbits (R) and goats.

Decision of the results of the tumor transplantation.

Criteria used by us about the results of the tumor transplantation were as follows:

1) **Death of tumor:** to die on account of the tumor growth with definite intervals after the transplantation.

2) **Natural healing:** to heal from tumor spontaneously with the abrupt degeneration and the disappearance of tumor cells in the abdominal cavity after approximately 10 days of continuing tumor growth (spontaneous regression).

3) **Relapse:** to relapse from metastatic focus after the natural healing in the abdominal cavity. Above mentioned 1), 2) and 3) are reckoned as positive transplantation.

4) **Imperfect transplantation:** the tumor proliferates weakly and disappears after 2-4 days.

5) **Negative transplantation:** rapid disappearance of the tumor without proliferation.

A, Successive transplantations to rats of Yamashita strain. (Table I)

When Yoshida sarcoma was transplanted successively into the abdominal cavity of the white rats of Yamashita strain, the tumor growth was active, infiltrating extensively into many organs and tissues, and was detected in the circulating blood in its last stage. All cases so far died of tumor within 10–13 days. When this tumor was transplanted in rats of the other strain, its proliferativity was still active in the earlier several generations. When the low proliferative tumor in animals of the different strain was transplanted in rats of Yamashita strain again, it recovered the proliferativity and in consequence the animals died of tumor.

Table 1. Number of generations 120. Total number of rats 300.
Death of tumor 300 (average day to death 10.5)
Natural healing 0.

	Average weight	Average days to death
Young rats	75 g	10
Adult rats	150 g	14

The tumor grew also intensively when transplanted subcutaneously or intracutaneously and animals died of tumor at last. Even in the cases of the intravenous

transplantation, the tumor grew extensively over all organs of the body of animals as if it changed into leukemia. In the last stage the tumor cell was found in the circulating blood. However, the affinity of tumor to bone marrows has not been proved to be conspicuous. The proliferativity of the tumor changed neither in its earlier generations nor later.

The results in the unclassified hybrid rats (S. G.) were as in Yamashita strain. And rats died mostly of tumor in a high rate but sometimes there was natural healing.

B. Successive transplantations to rats of Wistar strain (W_2). (Table 2)

When Yoshida sarcoma was transplanted successively into rats of Wistar strain in our Institute (W_2) from those of Yamashita strain or from the hybrid rats, the tumor grew actively without relation to the maturity of rats till almost 10 th generation and rats died of tumor after 13 days on the average.

But after about 10 th generation the growing tumor cells fell spontaneously into the vacuolar degeneration about 10–12 th day after the transplantation and they disappeared from the abdominal cavity. Besides, the metastatic tumors in subcutaneous tissues and in the other parts were almost reduced in size following the disappearence in abdominal cavity and fell into the cicatrization, and at last many cases showed natural healing. In these generations (5–15 gen.) adult animals (more than 100 g in weight) mostly showed natural healing and younger ones (less than 100 g in weight) died of tumor. But the proliferativity of the tumor was of less degree regardless of the maturity of rats during further generations, and after about 20 th generation tumor did not grow remarkably except in the abdominal cavity, and most of rats underwent natural healing on the 12 th day after the transplantation. When Yoshida sarcoma having a definite proliferative activity was transplanted simultaneously in some young and adult rats of Wistar strain the younger died mostly of tumor and the older healed out spontaneously. By transplanting successively in the younger Wistar-rats, they died of tumor extending over 10 generations, while in the older they healed out already from the earlier generation.

After 20 th generation this tumor had a tendency to show the type of natural healing and its proliferativity was now stabilized to some low rate. This low prolific tumor cells take generally large or anisocytotic cell form and light staining characteristic and their nuclear division displays a less activity. When these cells were returned to rats of Yamashita strain, they recovered their native proliferativities during several generations.

These facts show that such alteration of proliferativity of tumor cells is reversible but it is not reversed directly.

Table 2 Successive transplantations in Wistar strain (W_2). Succession 8 lineages, 100 generations. Total number of rats 900.

Generation	Death of tumor		Natural healing	
	Cases	Days to death	Cases	Days to healing
1 - 10	151	13	51	12
11 - 20	56	15	41	13
21 - 30	6	12	38	14
31 - 40	14	10 - 21	75	11 - 15
41 - 100	21	14 - 35	450	10 - 19

Correlation to body weight

Generation	Death of tumor		Natural healing	
	Average weight	Days to death	Average weight	Days to healing
1 - 13	86 g	13	128 g	10
14 - 43	60 g	17	136 g	12
44 - 100	83 g	14 - 35	114 g	11

The tumor transplantation to WY-F1.

When Yoshida sarcoma having a definite character—that is, death of tumor in Yamashita strain and natural healing in Wistar strain—was transplanted to rats of cross-bred of the 1st generation (F_1) offspring by the pairing of Wistar- and Yamashita strain (WYF_1), the tumor grew distinctly but the natural healing was found only in the abdominal cavity on the 11th day, while the subcutaneous tumor grew further and all the animals died of tumor on the 30th day after the transplantation.

On the contrary the cross-bred YWF_1 died of tumor in all cases.

This result shows that these cross-bred rats (WYF_1) are of a middle type between those of Wistar- and Yamashita-strain. It may be assumed that the death of tumor relapse under the similar formula sometimes happened in the cases of the unclassfied hybrid is owing to the factor dormant in the above described WY-F_1.

C. Successive transplantations to Water rats (D) (Table 3).

When Yoshida sarcoma with strong proliferativity was transplanted successively to Water rats, the rats died of tumor as well till the 10th generation, but with the Sarcoma of weak proliferativity natural healing occurred in the earlier several generations. But there were found two types, i.e., one is death of tumor and the other is natural healing and they irregularly mixed in the successive transplantations after 10th generation.

There are two kinds in the normal sera of Water rats: one of which (D_2) agglutinates lightly the Yoshida sarcoma cells, while the other (D_1) does not agglutinate them. When Yoshida sarcoma with a definite proliferativity was inoculated into these two kinds of rats, the rats having no agglutinins (D_1) died of tumor or showed natural healing after long periods, the rats that have normal agglutinins mostly showed natural healing within a relatively shorter time.

Table 3. Successive transplantations in Water rats (D).
Succession 3 lineages, 70 generations, 215 rats.

Death of tumor 55 cases		Average days to death	27
Natural healing 70 〃		Average days to healing	17
Accidental death 90 〃			

Water rats	Normal agglutinin against Yoshida sarcoma	Cases	Death of tumor	Natural healing
	(−)	27	17 (33 days)	10 (21 days)
	(+) (2−32X)	16	0	16 (15 days)

D. Successive transplantations to Roof rats (K) (Table 4)

In the cases of roof rats Yoshida sarcoma grew only in the abdominal cavity till about 8th day after the transplantation, and no infiltration was found in the surrounding tissues or organs. And all of rats up to the 50th generation showed natural healing after 8–12 days.

Table 4. Successive transplantations in roof rats (K).
Succession 4 lineages, 47 generations, total animals 135.

Death of tumor	0	
Natural healing	120	(8 - 12 days)
Accidental death	15	

E. Successive transplantations to Mice (Table 5).

Yoshida sarcoma is able to grow in the mouse only in the abdominal cavity. All of the transplantated mice showed natural healing on the 5–7 th day after the transplantation. But the successive transplantation was demonstrable more than 30 generations when the tumor cells were transmitted 24 hours before the regression, And then the reverse transplantation to the white rats was always possible. So it can be said that the heterogenetic transplantation of Yoshida sarcoma to mouse is successful.

Table 5. Successive transplantations in mice (M)
Succession 7, generations 30, cases 238.

Death of tumor	0	
Natural healing	192	(4.5 days)
Incomplete transplantation	46	

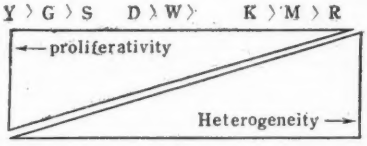
Summary on the successive transplantations to the several strains of rats.

It is summarized from the above-mentioned results that Yoshida sarcoma is

transmissible in rats to some considerable extent of strains. The proliferativity in the successive transplantation is the strongest in Yamashita strain, and almost equal in the unclassified hybrid, being lower in the order of Water rats, Wistar strain, Roof rats and mice. Tumor can hardly grow in rabbits.

It can be concluded that these differences in proliferativity are due to the difference of strains between the animals from which Yoshida sarcoma originated and the animals to which the sarcoma was transplanted.

Table 6 Proliferativity of Yoshida sarcoma transplanted in several strains of rats



1. Strains of death of tumor (Y. S. G)
2. Intermediate strains (D, W)
3. Strains of natural healing (K, M)

The animals can be divided in the following groups from the aspect of their behaviors toward Yoshida sarcoma:

1) Group of "death of tumor", 2) group of "natural healing" 3) group of "its mixed type."

That is, in the process of the successive transplantations, all dies of tumor without variation in the proliferativity in 1), all undergoes natural healing under the same condition in 2), and rats die of tumor in the earlier generation, then gradually inclines to the natural healing or the above two types of courses are mixed in 3). (Table 6).

Process leading to the natural healing

The problem is to ascertain the mechanism by which the natural healing takes place spontaneously during the proliferation of tumor. The process after the transplantation of Yoshida sarcoma was observed cytologically as follows.

a. Reaction in the first transplantation.

When Yoshida sarcoma was inoculated into rats, a slight reaction accompanied with leukocytes and monocytes was caused, reaching the maximal degree after 12 hours. This is the normergic reaction against Yoshida sarcoma as a foreign body. This reaction appeared very weakly and is a transient character in Yamashita strain and the hybrid, somewhat stronger in water rats and in Wistar strain, and still stronger in roof rats, mice and rabbits, which are heterogenous to animals in which the tumor originated. In roof rats and mice the reaction appeared through all the process of the tumor growth. It is considered that the

normergic reaction takes part in the inhibition against the tumor growth from the fact that this reaction to the heterogenous transplantation increased in inverse proportion to the tumor growth. (Table 7)

b. Behavior of tumor cells.

The cellular reaction in Yamashita strain or in the hybrid, in which the normergic reaction is very weak, disappeared soon. The transplanted tumor cells grew as if they were cultivated purely, and they extended into the tissues or in the circulating blood under the anergical state until the animals died of tumor.

The similar process was found in water rats and Wistar strain when Yoshida sarcoma with strong proliferativity was transplanted, while with the sarcoma of weak proliferativity, even in roof rats and mice, the tumor cells vanished suddenly with degeneration in about 10 days after a certain period of growth, and at the same time a strong cellular reaction recurred. In the degenerative form of these tumor cells, at first, neutral red granulas were shown distinctly by the supravital staining, became rough and dispersed gradually, and many small vacuoles appeared in protoplasms and finally the tumor cells fell into the vacuolar degeneration, fatty degeneration and cytolysis. At last, all tumor cells completely disappeared from the abdominal cavity adout 24 hours after the beginning stage of degeneration.

At the same time, metastatic tumors in the subcutaneous or other parts also diminished in size, degenerated and were absorbed in the same way.

These changes of the tumor cells were observed in all cases of the natural healing and were distinctly noticed, above all, in rats of Wistar strain.

c. The cellular reaction at the stage of the natural healing.

At the same stage when the above-mentioned degeneration began, leukocyts that were not noticed till then, emigrated again abruptly in the abdominal cavity accompanied with fibrin exudation. Subsequently, monocyte reaction took place of it and they decreased after the disappearance of tumor cells. This reaction shows a tendency to take an acuter and stronger phase compared with the normergic reaction in the earlier stage of transplantation. It is considered, therefore, that this reaction is the "primary allergic reaction" (Takeda) based upon the production of antibodies against Yoshida sarcoma, because the ordinary allergic reaction caused by foreign protein generally found in the abdominal cavity of rats coincides with this reaction (Table 7).

Table 7 a. Normergic and allergic reaction during the tumor proliferation.

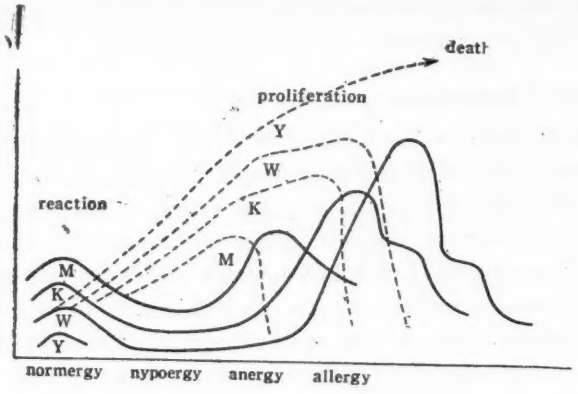


Table 7 b.

	Normergy	Anergy	Allergy
Death of tumor (Y. G. S.)	+	±±	±
Natural healing (W. D_2)	±	±	±±
Natural healing (K. M.)	±±	+	±
Negative transplantability (rabbit)	±±	−	−

Conditions of the death of tumor and the natural healing.

The following conditions are taken into consideration from the results of the transplantation as mentioned above.

The transplantated animals were the more heterologeous, the lower their prolific abilities became and the cases of the natural healing increased. The prolific ability of tumor decreased in consequence of repeating the transplantation successively in the cases of Wistar strain and Water rats, and most of these rats got the natural healing in the later generation. The young rats died of tumor and grown-up rats got the natural healing in Wistar strain, not only in the successive transmission, but also by transplantation in young and adult rats at the same time.

Besides, there were two types in normal sera of Water rats, one had no agglutinins against tumor cells and here the prolific ability of tumor was strong and the rats died of tumor, while the other having agglutinins against tumor got the natural healing. Roof rats had also normal agglutinins against tumor cells and got well.

It may be concluded that, the tumor growth is inhibited by means of normergic reaction, as far as the animals are lineally more heterogenous from the tumor, and moreover the above-mentioned other secondary inhibiting factors take part in this mechanism. But the noumenon of the natural healing which comes out

abruptly during the proliferation cannot be explained only by the decrease of the prolifelative ability of the sarcoma cells.

The such cellular reaction as the so-called "allergic reaction" was always noticed in the stage of the natural healing.

It is considered that several antibodies in animals develop against the complicated heterogeneity of tumor cells. This fact is found in the cases of the natural healing, as referred later as some immunological phenomena in this paper. On the other hand, this phenomenon can hardly be noticed in the cases of the animal's death of tumor. That is to say, the reduction of proliferativity and the generation of immune bodies are two important conditions for the natural healing of tumors (Table 8).

Table 8 Conditions between death of tumor & natural healing.

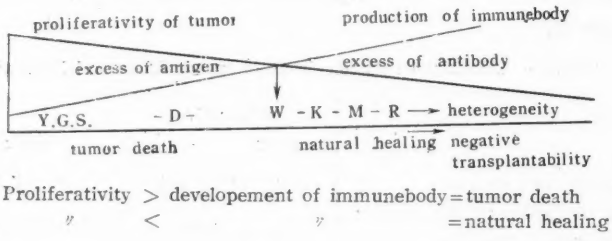
Condition	Proliferativity	
	strong	week
Heterogeneity of animals	low (Y. G. S. D.)	high (W. K. M.)
Decrease of proliferativity during successive transplantations	none (Y. S. G.)	+ (D. W.)
Grade of maturity of animals	jung (D. W.)	adult (D. W.)
Normal agglutinins	(−) (Y. G. S. W. D_1)	(+) (D_2. K.)
Production of antibody	low (Y. G. S.)	high (W. K. M.)
	tumor death	natural healing

The combination of these antibodies with tumor cells results in the degeneration and the disappearence of tumors cells and brings about the natural healing. The so-called "primary allergic reaction" in the form of the cellular reaction arises when antibodies in tissues and the component of tumor cells inter-act upon each other. These allergic correlations are understood under the idea, which is allowed as the similar one in germ infection.

Generally speaking, the tumor grows increasingly and the production of antibodies is scanty against it when it is transplanted into animals of the lineage in which the tumor originated; while in animals of the distant lineage, the proliferativity is weak and antibodies are produced in a large amount. On the other hand, it is observed that animals die of tumor showing no immunity, if high potency tumor was transplanted into rats of Wistar strain, while with low potency tumor, Wistar rats show the natural healing leaving immunity. As it is hardly considered that the proliferativity of tumor has direct influences upon its antigenicity, tumor cells in a large quantity growing with high prolifelative ability absorb more and more antibodies, and they finally induce animals to tumor death in a condition of antigensurplus leaving no antibodies. On the other hand, tumor cells growing with low proliferative ability absorb large amounts of antibody and lead animals

to the natural healing still leaving large amounts of antibody. Namely, the condition of antigen-surplus brings the death of tumor and of antibody-surplus results in the natural healing. The tumor death or natural healing is decided according to the correlation between the prolifelative ability and the production of antibody. It is assumed that these correlations are proved not only in the heterologeous transplantation as in Wistar rat, but also in the iso-transplantation to the same lineage from which the tumor generated and perhaps in animals with original tumors it can exist in masked phase (Table 9).

Table 9



Proliferativity > developement of immunebody = tumor death
〃 < 〃 = natural healing

The nature of Yoshida sarcoma and its relation to leukemia.

a. The lineage of the matrix from which Yoshida sarcoma generated.

Yoshida sarcoma generated in one of white rats of the unclassified hybrid. According to the results in our laboratory, the proliferative ability was the strongest and animals died of tumor in 100% in the rats of Yamashita strain. The heterogeneity and the production of immune bodies are very weak and low in this strain, and moreover, as mentioned later, when Yoshida sarcoma was transplanted into a rat, whatever the strain, it has an anitigenicity most common to normal nucleated cells of rats of Yamashita strain, and these immune bodies against Yoshida sarcoma can be almost all absorbed with cells of Yamashita strain.

These facts suggest not only that the lineage of matrix of these tumor cells is of a rat of an unclassified hybrid which has a disposition belonging to Yamashita strain, but also that the normal cells of rat in which the sarcoma was transplanted do not change directly into tumor.

It is considered, therefore, that the attitude of the transplanted tumor in Yamashita strain should accord with the course of the tumor growth after original generating in a rat (Table 10).

Table 10. Matrix of Yoshida sarcoma.

Proliferativity	Y > S > D > W > M > R
Heterogeneity (normergic reaction)	Y < S < D < W < M < R
Production of immunebody	Y < S < D < W < M < R
Common antigenicity to Yoshida sarcoma.	Y > S > D > W > M > R

b. Matrix cell of Yoshida sarcoma.

Prof. Yoshida regarded the original cells of Yoshida sarcoma as monocytes in the abdominal cavity, because the following characters were also observed in Yoshida sarcoma; i. e., notch of nucleus, azure granula in cytoplasm, granules in supra-vital staining, and phagocytosis which are the characteristics of monocyte.

It is proved further in our laboratory that the degenerative forms of Yoshida sarcoma cells due to cyto- or nucleotoxin such as nitrogen mustard, urethan, colchicine, etc., were most similar to those of common monocytes. The monocytes were, however, apt to degenerate earlier than Yoshida sarcoma cells.

As mentioned later, rat serum of Wistar strain which was obtained after the healing from Yoshida sarcoma lets Yoshida sarcoma cells degenerate under the vacuolar form and also agglutinate them. This serum reacts upon monocytes in the abdominal cavity of Yamashita strain entirely in the same way, but it has no influence upon erythrocytes or on the other cells of Yamashita strain.

The above-noted observations indicate, therefore, that there exists the specific antigen common between monocytes of Yamashita strain and Yoshida sarcoma.

It must be concluded from the morphological, functional and antigenic correlations that Yoshida sarcoma generated from the so-called monocytes found in the abdominal cavity of Yamashita strain and still possesses the characteristics belonging to monocyte of Yamashita strain even after being changed into tumor, and, even though transplanted into any strain or animal.

If Yoshida sarcoma has characteristics of monocytes, there arises the question of how to make clear the relation to leukemia.

Yoshida sarcoma cells transplanted into the abdomen in Yamashita or non-classified hybrid appeared in circulating blood in the last stage when animals lose their normal reactivity against tumor cells, increasing rapidly at the agonal period.

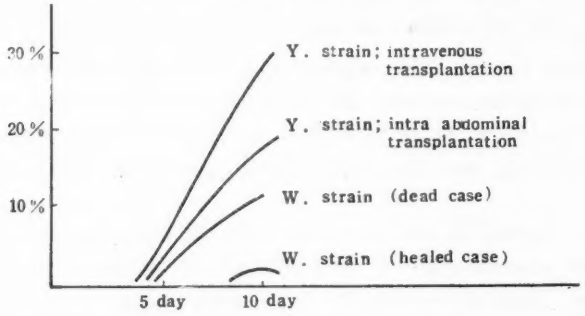
The total numbers of leukocytes in blood were 20,000~30,000 and the ratis of Yoshida sarcoma cell against leukocyte was 5-20% in the peripheric blood and 20-30% in the heart blood.

In the cases of the intravenous transplantation in Yamashita or the hybrid strain, tumor cells disappeared trasiently from the blood but they appear again in the last stage reaching more than 30% of leukocytes, because of the increasing

infiltrative activity in organs as in leukemic infiltration. When foliamin, a derivative of the folic acid and vitamin C were administered to rats, tumor cell numbers in blood were apt to increase more than ever.

On the other hand, in the cases of Wistar rats in which Yoshida sarcoma grows poorly the sarcoma cells were hardly seen in the circulating blood (Table 11).

Table 11. Yoshida sarcoma cells in circulating blood.



In conclusion, tumor cells do not appear in the circulating blood under the state of high normergic or allergic reaction, but they appear only in the anergic situation.

As most of the tumor cells in blood show, however, several degenerative forms without mitosis, so the tumor cells can never grow in blood, while out of the blood vessels they are able to proliferate easily. All tumor cells in blood fall immediately into degeneration and necrosis.

In vitro, Yoshida sarcoma cells can survive in normal ascites or in salt solution for a long time and are also transplantable, but when soaked in the serum they degenerate more and cannot be transplanted.

Namely, normal blood is not a suitable milieu as compared with ascites to maintain life of Yoshida sarcoma. Consequently, the appearance of sarcoma cells in blood is not caused by the active invasion on account of having an affinity for blood, but it is rather a process leading to degeneration of tumor cells around blood vessels passively forced out into the circulating blood, because the blood vessel wall loses its resistance under the anergic situation in the last stage.

There may be several conditions under which bacteria found in tissues appear in the circulating blood in the cases of germ infecction. The increase of germs in blood in the last stage of infection is an anergic sepsis, being due to the loss of reactivity. The phenomenon of Yoshida sarcoma cells appearing in the circulating blood in the last stage should be considered as similar to the anergic sepsis.

And the similar observation should be also noticed in the appearance of human leukemic cells in blood. As Yoshida sarcoma is a kind of monocytoma because of having monocytic characters, it is like the monocytic leukemia when it appears

in the circulating blood. It has been affirmed by many authors that the monocytic leukemia can be found in man, and there still remain problems to find a direct agreement of the character of Yoshida sarcoma with that of the human monocytic leukemia.

Immunity and allergy in the transplantation of Yoshida sarcoma

It was mentioned before in this paper that the tumor cells in Wistar rats degenerate and disappear and animals show the natural healing in consequence of the primary allergic reaction caused under the condition of a definite balance between the proliferative ability of Yoshida sarcoma and the production of immune bodies against it. A further investigation of this phenomenon will give an important key that serves to disclose the enigmatic nature of the immunity against tumor, a problem much discussed to-day.

a. Immunity and allergy in the retransplantation.

When Yoshida sarcoma was retransplanted subcutaneously after abdominal transplanting in rats of Yamashita strain or the hybrid the tumor cells grow easily. But they could not grow finally either in the abdominal cavity or in the subcutaneous part for over 40 days, if the subcutaneous transplantation and the excision of tumor were repeated by turns.

The tumor growth in the cases of the subcutaneous transplantation in Wistar and roof rats was generally feeble, but the retransplantation into the abdominal cavity later than the 6th day after subcutaneous transplantation showed negative sign for more than 50 days.

In the cases of Wistar rats, water and roof rats that healed spontaneously from Yoshida sarcoma, the retransplantation abdominally or subcutaneously and intravenously resulted in negative for over 100 days.

When retransplanted immediately after the natural healing in these cases, there occurred leukocytic and monocytic reactions rapidly accompanied with fibrin exudation, and tumor cells disappeared with vacuolar degeneration within 6–12 hours.

The cellular reaction decreases in animals which underwent natural healing more than one month before, and it shows nearly the normergic situation and tumor cells decrease slowly when they were retransplanted into animals in which natural healing took place over three months before (Table 12 a).

The phenomenon of resistance against this retransplantation is the same as the primary allergic reaction in the natural healing. This phenomenon is the so-called "secondary allergic reaction" (Takeda).

When Yoshida sarcoma cells were reinjected intravenously in the cases of naturally healed rats, they are caught rapidly in the capillary blood vessels of

lung in larger quantities as compared with control rats and there occurs the allergic reaction accompanied with haemorrhage and embolism and tumor cells degenerate soon. This correlation accords, in short, with the allergic reaction caused by germs (Table 12 b).

Table 12 a. Retransplantation of Yoshida sarcoma.

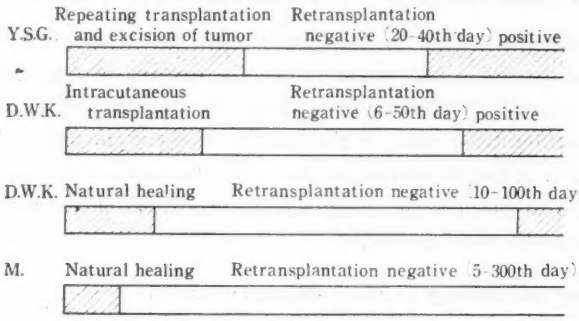


Table 12 b

	Days after natural healing (W)	1-10 days	10-30	30-90	90-
retransplant.	No. of hours in which tumor cells vanished.	6-12 hours	24	72	120
	Cell reaction (secondary allergic reaction)	##	#	+	+

b. Injurious action of the immunized sera and ascites upon tumor cells in vitro.

It is considered from the above-observed facts that the body fluid of the healed rats contains the antibody against tumor. When Yoshida sarcoma cells were soaked, in vitro, in ascites or in sera of the healed Wistar rat by a definite method, all tumor cells fell into the vacuolar degeneration within 6–12 hours as observed in the cases of the natural healing. The injurious action was stronger in sera than in ascites and was observed in the former in 80–160X of dilution and it was observed in rats healed spontaneously over 100 days before. This action upon tumors was the strongest, above all, in Wistar strain and lower in water and roof rats.

The sera of rabbits and goats immunized against Yoshida sarcoma had more intensive action, and then they agglutinated and fused all kinds of rat-cells as well as sarcoma cells within one hour after soaking.

c. Neutralization by means of the healed sera of rats.

The healed sera were diluted in different concentrations and Yoshida sarcoma cells were mixed for 5–10 minutes in vitro. When this mixture was inoculated into rats of Wistar strain, the negative transplantation was proved in Wistar rat

sera upto 5X of dilution, in those of water rats in 2X of dilution, in roof rat in original sera and in rabbit immune sera in 1–5X of dilution, but the sera of Yamashita strain and Wistar strain which died of tumor could not inhibit the transplant.

d. The passive immunization by means of sera immunized against tumor.

The healed rat sera were injected into the abdominal cavitiy of a normal rat and after a definite interval tumor cells were transplanted. The transplantability of the tumor was inhibited in all cases, though the results were influenced by the methods of previous administration of serum : i. e. the sort and quantity of serum, the lineage of transmitted animals and intervals before the subsequent tumor inoculation. For instance, the tumor transplantation was unsuccessful even 3 days after inoculation of the healed sera (0.5 cc) of Wistar strain into the abdominal cavity of the same strain, while it became positive after about 24 hours, if the serum was that of water or roof rat.

Namely, serum potency to neutralize the tumor cells in vivo, stands higher in the order as follows: W>K>D>Y. Restrainning ability is most remarkably noticed in rats of Wistar strain as animals used for the transplantation, while it needs larger amounts of healed serum to repress the transplantation in the cases of Yamashita or the hybrid stain; i. e., W>K>D>Y (Table 13).

Table 13. Passive immunity against tumor with immune sera.

Serum	Dosage	Transplanted lineage	Days after injection	Result of transplantation
W	0.5 cc	W	3 days	—
K	0.5 〃	W	1	±
D	0.5 〃	W	1	±
Y. G	0.5 〃	W	1 hour	+
W	0.1 〃	W	1 day	—
W	0.5 〃	K	1	±
W	0.5 〃	D	1	+
W	3.0 〃	Y	1	+

e. Agglutination test of tumor by means of sera immunized against tumor.

Although the complement fixation test has been used by many authors as the immunological reaction of tumor, the agglutination test is also available for this purpose, because Yoshida sarcoma cells react as keenly as the common blood cells.

Normal sera of Yamashita and the hybrid rats have no agglutinins and the aggutination test results almost in negative not only in the case of tumor death, but also if a definite resistance against tumor was obtained by repeating trans-

plantation and enucleation in the skin.

But in hybrid rats the test showed sometimes 32X agglutination titers after transmission of tumor.

Wistar strain has also no normal agglutinins. When Yoshida sarcoma with high proliferative ability was transplanted, rats died of tumor without appearance of agglutinins, while with low potent sarcoma, sera of rats showed 32X titers in the test during about 5 days, and their titers rose gradually up to 640X in maximum within one week after the healing and then they fell after about 50 days.

These sera agglutinate equivalently the abdominal monocytes of Yamashita strain as well as Yoshida sarcoma cells, but do not agglutinate erythrocytes of Yamashita strain.

Water rats having no normal agglutinins (D_1) died of tumor without ascending titers, even if tumor was transplanted; while those having normal agglutinins (D_2) got well with ascending titers to 640X. Roof rats have normal agglutinins and got well with ascending titers to 1280X. Sera of rabbits or of goats immunized against tumor showed 320–2000X titers; besides, they agglutinated strongly all rat cells of any lineage. They have, namely, the strong species-specificity.

But as will be mentioned later, when anti-rat factors of sera of rabbits were absorbed by erythrocytes of Yamashita strain, agglutinins against erythrocytes were deprived, while there still remained the agglutinins against tumor and monocytes. Absorbing with normal tissues of Yamashita rats or tumor tissues agglutinins against tumor and monocytes were deprived completely.

The above-mentioned anti-transplantability, neutralization, passive immunization, injurious action and agglutination test, which were tested with the immunized

Table 14. Agglutination test on Yoshida sarcoma rat serum transplanting Yoshida sarcoma.

Strains of rats	Agglutinogen				Outcome
	Tumor (before)	Tumor	Monocytes (Y)	Erythrocytes (Y)	
Y S. G	0	0	0	0	tumor death
		0	0	0	〃
W	0	0 640	0 320	0 0	tumor death natural healing
D_1	0	0	0	0	tumor death
D_4	32	640	640	0	natural healing
K	4	1280	1280	0	natural healing
R	4	2000	2000	2000	3–4X immunization

animals and their sera, showed nearly parallel correlations, but a certain variation among each potency was found according to each experimental method. It is considered, conclusively, that each immunological phenomenon is a partial expression of the complicated factors which caused the immunization against Yoshida sarcoma, containing species or strain specific and organ specific antigens as a definite tumor (Table 14).

The noumenon of immunity against Yoshida sarcoma.

A living body must have some antigenic difference among its body fluid, non-nucleated and nucleated tissues besides the species specific antigen.

Yoshida sarcoma has naturally not only the species-specific antigenicity always common to the animal from which it generated, but also the antigenicity common to monocytes which may be the matrix cells of Yoshida sarcoma, besides the common antigenicity to the nucleated cells of this strain. Furthermore, a specific antigenicity can be also assumed in these cells because tumor-specific protein, enzyme or virus, etc. must be concealed in these cells which mutated to tumor.

A. Species-specific immunity

If the immunization against Yoshida sarcoma is the species-specific one, being common to cells of the animal in which it generated, antibodies against tumor should be produced by immunizing against normal tissues of the generated animal instead of using Yoshida sarcoma. In fact, the sera that were obtained by the nutual immunization among Yamashita-, the hybrid strain and Water rats which are genetically closely related to Yoshida sarcoma agglutinated neither Yoshida sarcoma cells nor monocytes of immunized animals and the antitransplantability was also not revealed.

On the other hand, the sera which were obtained from animals distantly related, i. e., Wistar and roof rats, mice and rabbits immunized against fresh embryo, kidney, liver, monocytes or erythrocytes of rats closely related to Yoshida sarcoma (i. e., Yamashita- and the hybrid strain and water rats), agglutinated monocytes and erythrocytes of animals used for immunogen as well as Yoshida sarcoma in some degree.

And in these immunized animals (W. K. M) the transplantation of Yoshida sarcoma was prevented during from one to two weeks after immunization. Its antitransplantability was not, however, so strong as to cause the degeneration of the tumor after three weeks. The immunization against killed normal tissues or against sera of these rats resulted almost in the negative antitumor immunity.

On the contrary, when rats closely related to Yoshida sarcoma were immunized against tissues of rats distantly related (Wistar- and roof rats and mice), the sera agglutinated cells of animals used as immunogen, but not Yoshida sarcoma cells.

The antitransplantability against Yoshida sarcoma was not evoked in these animals.

These sera that agglutinated Yoshida sarcoma could be absorbed by adding normal rat tissues used as immunogen, and they agglutinated neither rat cells nor Yoshida sarcoma cells, and did not injure the latter. In short, the immunization against Yoshida sarcoma is partially due to the species-specific factor common to the normal nucleated rat cells, and this factor can also be absorbed by normal rat cells.

The more distant the lineage of animals used for immunization are from Yoshida sarcoma, the more distinctly are produced its antibodies, which injure not only rat cells but also Yoshida sarcoma (Table 15).

Table 15. Species specific immunity.

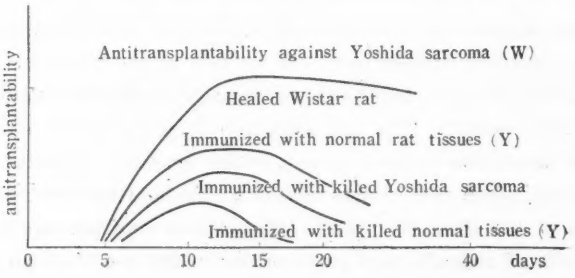
Immunized animals	Tissues immunizing animals*	Agglutination			Antitransplantabilites
		Tumor	Monocytes	Erythrocytes	
Y	D.W.K	0	0	0	0
D	Y.W	0	0	0	0
W	D.Y	±	+	0	+
K	Y.W	+	+	+	+
R	Y.W.K	++	++	++	

* Tissues as immunogen: normal tissues of rats (liver, kidney, spleen erythrocytes etc.)

B. Immunity against nucleated cells of rats.

In contrast to the above-mentioned facts that the transient antitransplantability continued weakly only for 1–2 week when distantly related rats (W. K) were immunized against near-related nucleated rat-cells (Y. D), the antitransplantability continued strongly for more than 100 days when rats underwent the natural healing from Yoshida sarcoma. But animals showed nothing but the speciesspecific immunity transiently as in the case of immunization against normal rat tissues of nearer lineage, when they were immunized against the killed Yoshida sarcoma cells. This fact indicates that the quantitative and temporaly differences are

Table 16. Antitransplantation of immunized rats (Wistar).



remarkably formed in each immunization between normal rat tissues and living Yoshida sarcoma cells.

The serum of Wistar rat that healed naturally agglutinates both Yoshida sarcoma and monocytes of Yamashita rat, but not erythrocytes of the latter.

The serum of Wistar rat immunized against monocytes in the abdominal cavity of Yamashita rat agglutinates both monocytes of Yamashita rat and Yoshida sarcoma, but not erythrocytes of the former (Table 16).

Conversely, the serum of Yamashita rat immunized against monocytes of Wistar rat agglutinated monocytes of Wistar rat, but not Yoshida sarcoma or monocytes of Yamashita rat.

It is, therefore, supposed that there exists at least a common nucleated-cell-tropic antigen between Yoshida sarcoma and abdominal monocytes of Yamashita rat.

When large amounts of the serum of healed Wistar rat were administered, the injurious potency against Yoshida sarcoma increases but the toxicity against rat cells still remained low, while administered the diluted sera of rabbit immunized against Yoshida sarcoma, the injurious effect against tumor cells decreased, but the toxicity against rat cells remained still remarkably. Namely, the injurious action against both tumor and rat cells, which are qualitatively different from each other, should be mingled in different proportions in these sera. Now, when the sera of rabbits and goats immunized against Yoshida sarcoma were absorbed with erythrocytes or with nucleated cells of Yamashita rat, i. e., liver, kidney, etc., the agglutinative titer of the fomer showed zero only against erythrocytes, and of the latter also negative against erythrocytes, nucleated cells of rats and sarcoma cells.

And the injurious activity against rat decreased remarkably, while the injurious factor against tumor remained still more active. Freeze-dried Yoshida sarcoma was deprived of its powerful antigenicity as tumor, and it showed only its species-specific antigenicity as tumorigenic rat cells. When the sera of healed Wistar rat or of rabbits and goats immunized against Yoshida sarcoma were absorbed with these frozen Yoshida sarcoma cells, they did not agglutinate entirely Yoshida sarcoma as well as erythrocytes and monocytes of Yamashita strain. They did hardly injure rat cells, but they still had more injurious activity to cause the tumor to fall into the vacuolar degeneration in vitro or in vivo. But, when these sera were absorbed sufficiently with native Yoshida sarcoma, the injurious factors against Yoshida sarcoma decreased remarkably.

It should be recognized from these facts that the results of agglutination test are due to species-specificity and at least nucleated-cell-tropic antigen of rat, and the real resistivity against tumors should lie out of this factor. At the same time, these facts show that there are antibodies of different characters in rabbit

sera immunized against normal tissues and native tumor cells of rats (Table 17).

Table 17. Agglutination test with immunized sera.

Sera	Absorption with	Agglutination test with erythrocytes (Y)	〃 with monocytes (Y)	〃 with Yoshida sarcoma	Histological changes of Yoshida sarcoma vacuolar Deg.	cytolysis.
Healed rat (W)	/	0	++++	++++	++	±
〃 (K)	/	0	++++	++++	++	±
Immunized rabbit	/	++++	++++	++++	++++	++++
〃	Erythrocytes (Y)	0	++++	++++	++	±
〃	Liver, kidney (Y)	0	0	0	++	±
〃	Killed Yoshida sarcoma	0	0	0	+	±
〃	Native Yoshida sarcoma	0	0	0	±	−

As observed above, to summarize, there are the following facts.

1) The antibodies that are effective upon the tumor are absorbable with native tumor cells, in vitro, but not with killed sarcoma cells or with normal rat cells.

2) If native tumor cells are abundantly administered to animals healed naturally, they are desensitized, in vivo, and the retransplantation becomes possible transiently.

3) In the cases of transplantation with tumor of high proliferative power to Wistar rats, they die of tumor without leaving any immunity, while with that of low proliferative power, they show natural healing with immunity.

4) Even in the cases of Yamashita rats, from which sarcoma might have generated, they can obtain the immunity by appropriate methods somehow.

From these facts it can be presumed that some antibodies in some quantity may be produced not only in the transplanted rat of tumorigenic lineage, but also, perhaps, in the individual from which tumor originated. Nevertheless, these antibodies disappear after ceaseless absorption or neutralization by tumors which develop more actively, and the immunity aginst tumors may exist in a masked condition.

The above-noticed three specificities (against tumor, nucleated cells and species) are summarized as follows:

Anti-tumor factor in the serum of Yamashita rat without showing any agglutination and other reactions is probably due to the real tumor-tropic immunity, and sera of Wistar rat, rabbits and goats absorbed with killed tumor cells or with normal rat cells come under this category.

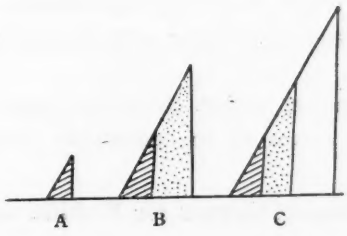
The serum of healed Wistar rat contains at least the organ- nucleated-cell-tropic factor agglutinating monocytes of Yamashita rat, besides tumor-tropic one. The sera of rabbits and goats absorbed with erythrocytes resemble the former.

The immune sera of rabbits and goats contain, moreover, plenty of species-specific antibodies against rat.

These correlations are shown in Shemata A, B and C. It is considered that these complicated factors must have influences upon the rat tissues or the tumor in each different potency (Table 18).

Table 18.

	Tumor-tropic	Nucleated cell-tropic	Species spec.
(A)			
Immunized sera (Y) against tumor by repeated transplantation and excision	+	0	0
Immunized sera of Wistar rat & rabbit absorbed with killed Yoshida sarcoma or with normal organs of Yamashita rat	+	0	0
(B)			
Sera of naturally healed rats (W).	+	+	±
Rabbit and goat sera immunized against Yoshida sarcoma absorbed with rat erythrocytes	+	+	±
(C)			
Rabbit and goat sera immunized against Yoshida sarcoma	+	++	+++



C. Quality of tumor antigens: (Antigenicity of tumor)

The strong antigenicity of Yoshida sarcoma was displayed to Wistar rats or rabbits only in the transplantation with living tumor cells, while the tumor cells treated with ultra-violet ray or by freezing at -60° for a short time had still some antigenicity, and by means of a short-term neutralization with immune antibodies or by soaking in formalin the antigenicity was weakened. And only the transient, weak species-specific antigenicity remains if the tumor cells were killed under a long-time freezing, under fusion or drying after freezing.

In short, the characteristic antigenicity which Yoshida sarcoma possesses con-

sists only in the living cells and it is so labil as to be denaturalized when exposed outside of living cells.

It may be assumed that this speciality is closely connected with the factors to let tumor cells live or grow, but it is a problem in future to be decided whether this would be a specific protein, enzyme or masked virus, which may be supposed to be related with this tumor (Table 19).

Table 19. Antigenicity of Yoshida sarcoma

Condition of Yoshida sarcoma inplanted in Wistar rat.	Antitumoric antitransplantability.	Species specific antitransplantability.
Native	±±	±
Ultra violet ray	±	+
-60°C (10')	+	+
Freezing	±	+
Repeated freezing and melting	−	+
Freezing-drying	−	+
Formalin	−	+
Other chemicals	−	±

D. Quality of antibodies.

Antibodies against this tumor have a cytotoxic character and consist of complicated mixtures—species- and nucleated celltropic, and perhaps tumor-tropic factors. They consist naturally of serum globulin. According to Tiselius's method, generally γ-globulin is rather decreased and α-and β-globulin increased in rabbit immune sera containing tumor- and species-specificities, and only α-globulin is increased distinctly in Wistar rat immune sera containing tumor- and nucleated cell-tropic factors.

It may become an important subject to extract some substance which affects only tumors specially, by means of an appropriate absorption method, in the future.

Immunological therapy for Yoshida sarcoma.

From the above-described facts the immunological therapy was attempted in our laboratory.

A. Therapy for the transplanted tumor in Wistar rats.

When the serum of healed Wistar rat was administered into the abdominal cavity of rats of the same lineage in which 48–72 hours before Yoshida sarcoma cells with so high prolificity as to let animals die of tumor have been transplanted, and in which tumor cells were growing as pure culture, decreased the tumor cells in the cavity during 6–24 hour after injection accompanied with the abrupt, vacuolar degeneration as in the cases of natural healing. The relapses were, however,

found sometimes, and by divided injections of the same serum (4–8 cc) for 4 days or so, animals got well perfectly without death from relapse.

Besides, water rats and roof rats transplanted with tumor cells could be cured also by the sera of the same healed source. When the inactivated (in 56°C 30') serum of rabbit immunized against tumor was administered into the abdominal cavity of Wistar rat transplanted 48–72 hours before, tumor cells fell into cytolysis and ascites became watery. But relapses were still found in some cases and by repeating the serum injections rats got well after 4 days or so. And yet some rats died of intoxication. Control rats (without receiving therapy) almost all died of tumor. Therapy for tumor-bearing water and roof rats was also possible by means of rabbit immune sera. However, as Wistar, water and roof rats are originally heterologous somewhat to animals in which the tumor originated, the natural healing is sometimes observed, so it is improper to discuss the therapy for Yoshida sarcoma only by the above-mentioned facts.

B. Immunological therapy for Yamashita and the hybrid rats.

These lineages are the original stem from which Yoshida sarcoma generated and all die of tumor regardless of the conditions of tumor transplantation.

When the serum of healed Wistar rat (2–3 cc) was administered into the abdominal cavity of these lineages in which tumor cells were transplanted 48 hours before and were growing as pure culture, tumor cells decreased in number accompanied with the vacuolar degeneration, but relapses came sooner or later. Even though the sera were injected in divided doses (ca. 10 cc in tolal), animals died of tumor after all.

Hence the sera of healed Wister rat were administered in large quantities in a single dose to an animal 48 hours after transplantotion, and then tumor cells decreased in number showing crisis on account of the strong vacuolar degeneration 2–3 days after. By repeating the injection of larger amounts of serum against the survived resistant cells in the relapse period, all cases could reach the perfect cure by using in all 50–70 cc of immune sera of Wistar rat after 4–7 days. The inhibiting effects against retransplantation were observed for 40 days thereafter in these four cases, but their sera showed zero titer in the agglutination test.

Being different from the rabbit immune sera, this rat serum did not injure the animals at all, except the slight degeneration of monocytes in the abdominal cavity, in spite of using such a large quantity of the serum in short term. In short, the healed serum of Wistar rat was most effective, but a difficult point is that the sera of twenty Wistar rats naturally healed are needed in order to cure only one Yamashita rat.

The serum of rabbit immunized against Yoshida sarcoma gives a strong, cytotoxic injurious effect upon Yamashita rat tissues as well as Yoshida sarcoma

cells. By administering native or inactivated sera the tumor cells disappeared immediately after degeneration, but at the same time all animals died of intoxication resulting in the degeneration and the necrosis of liver, kidney, spleen, etc., and also in agglutination and in lysis of erythrocytes.

Moreover, by injecting a small amount of the serum or the diluted serum so as to avoid the death from intoxication, no case could be saved from the death from relapse. Meanwhile, it was easy somewhat to cure perfectly by using the healed Wistar serum in order to prevent the relapse of animals in which a small dose of the immune rabbit serum was previously administered to cause the primary crisis.

As the sera of rabbits or goats immunized against sarcoma agglutinate and dissolve rat blood cells intensively, these toxic factors of the sera were absorbed sufficiently with rat blood cells so as to make titer zero and the sera already absorbed were injected into the abdominal cavity of Yamashita or the hybrid rats 48–72 hours after sarcoma transplantation, and then tumor cells decreased also critically under the vacuolar degeneration after 2–3 days. Some doses of the serum were added moreover to prevent the relapse and most of the cases under treatment were perfectly cured after 5–14 days. But the death from intoxication or from relapse was still observed.

It is confirmed in our laboratory that the perfect cure of Yamashita and the cross-bred rats suffering from Yoshida sarcoma, which has been very difficult to be cured, could be successful.

Then, attempts were made to study by means of comparative method between the injurious action against rat tissues and the therapeutic effects upon Yoshida sarcoma by the immune sera which were absorbed with several kinds of rat tissues and cells.

As it is supposed, the sera of rabbits and goats immunized against Yoshida sarcoma contain antibodies having a common efficiency upon the normal nucleated rat cells, these sera were absorbed with liver and kidney tissues of normal Yamashita and the hybrid rats and then they were administered in tumor-bearing rats. Their toxicity against rat decreased remarkably compared with sera not absorbed, and the acute cytotoxic action upon Yoshida sarcoma cells decreased and the cells vanished gradually within 4–7 days. Nevertheless, many cases fell as before into death of intoxication after the abdominal tumor had vanished.

When the immune sera were absorbed with erythrocytes and emulsions of kidney and liver, these sera did not agglutinate blood cells, but Yoshida sarcoma cell in a slight grade. When these sera were administered in to the tumor-bearing rats, the tumor cells disappeared from the cavity within 2–3 days, but animals sometimes died of intoxication after the disappearance of tumor.

It has been mentioned before that the tumor tissue preserves the species-

specific and nucleated cell-tropic antigenicity as a tissue of rat lineage from which the tumor generated, and loses its strong specific antigenicity, when Yoshida sarcoma was exposed repeatedly for a long time to the freezing and melting procedures. Absorbing the immune serum of rabbit with these treated tumor antigens, the normal species-specific and nucleated cell-tropic factors belonging to the original tumor animal will be theoretically absorbed perfectly.

As a matter of fact, these absorbed sera did not agglutinate entirely both Yoshida sarcoma and normal cells (erythrocytes and monocytes) of Yamashita strain. When they were injected into the abdominal cavity of Yamashita and the hybrid rats 48–72 hours after the tumor transplantation, the tumor cells decreased rapidly under the vacuolar degeneration and disappeared thoroughly from the cavity accompanied with the cellular reaction.

Even if in the cases of subcutaneous administration with these sera, the tumor cells disappeared from the cavity under the obvious vacuolar degeneration as in the cases of natural healing in Wistar rats, though it required more time and doses. But relapses and toxic death could not be avoided at definite intervals after the disappearence of tumor cells. We are now studing again how to absorbe the toxicity in the serum.

On the contrary, the serum absorbed with the native sarcoma cells, instead of the one subjected to freezing and melting, was decreased in its anti-tumor-specificity but the treatment did not succeed.

In the sera of rabbits immunized against erythrocytes, liver or kidney of normal Yamashita rats, the injurious effects on the tumor cells based on the species-specificity were also observed in some degree, but the toxicity against rats remained at the same time, and when the latter was absorbed, the injurious action upon the tumor cells also descended so low that no influence upon the tumor cells could be observed. Using the immune serum of goat that contains Forssman's antigens, instead of the rabbit immune serum, the therapeutic effect of the former agreed with that of the latter.

The histological observations about the influences of these sera upon the living body of rat, which will be described in detail in an other paper, were as follows:

1) Unabsorbed rabbit's sera: severe hyperemia, congestion, embolism and necrosis of tissues (liver, kidney, spleen, etc.) of rats were revealed under the influence of the antibody based on a strong species-specificity.

2) Absorbed sera: the injurious action fell down after absorption and the allergic changes decreased, while degeneration and swelling remained in Kupffer's cells of the liver and in pulpa cells of the spleen.

It is an observation of interest that in the case of absorbing with killed tumor cells the injurious factors fell down more obviously, and swelling and degeneration in Kupffer's cells could be scarecely found, but the degeneration in pulpa cells

of the spleen still remained.

As observed conclusively on the immunological therapy for Yoshida sarcoma, the serum of Wistar rat naturally healed is most favourable, while the sera of rabbits and goats immunized against tumor have also a therapeutic effect at least in the earlier stage of transplantation, if its species specific antibody against rats was absorbed completely by means of an appropriate method, but animals often have a tendency to die of intoxication after the disapperence of tumor cells. In order to prevent the emaciation during treatment must be considered the methods of absorption and of therapy as well as general care.

Generally speaking, the therapy becomes far more difficult when tumors were transplanted to rat lineages which are closely related to the original tumor animal, or when the intervals were prolonged after the inoculation of tumor cells.

As the tumor grows very actively and infiltrates into the surrounding tissues in the cases of rats closely related to the original tumor animal it needs a large dose of antibody to cure and at the same time the sera displays the toxicity upon the living tissues in which a common antigen with tumor remains, not being completely absorbed some time.

On the other hand, the immune serum of Wistar rat indicates chiefly an effective factor upon the tumor without injuring the animal.

There are numerous chemotherapeutic researches on tumors at present. There is little difference in the resistance against these chemicals between normal tissues and tumors. The problem in point is whether the anti-tumor factor (which is supported in our laboratory) is of a real specificity or not. But, if the real specific antibodies against tumors could be separated, it can infiltrate more easily into tissues and can affect tumors selectively without damaging normal tissues, being different from those chemical substances.

One of the factors which made the immunological therapy succeed in our case of Yoshida sarcoma is owing to the character of this tumor, which is an ascites tumor, affording rapidly a direct influence of effective substances upon each several cell of this tumor—a condition not prevailing in solid tumors.

Summary and Conclusions

It is necessary to select a suitable tumor and a pure strain of animals in order to study "the allergy and immunity against tumor."

Fortunately we could find some pure strains of rats and a ascites tumor of rat (Yoshida sarcoma), being appropriate for the delicate observation on this study.

During the successive transplantation of this tumor in rats we could learn the facts that the various attitude to Yoshida sarcoma among strains of rats, namely death of tumor (Yamashita strain etc.) and natural healing (Wistar strain etc.), is almost due to the genetical difference of strains between animal from which

Yoshida sarcoma generated and animal in which this tumor was transplanted. The natural healing, followed by the vacuolar degeneration of tumor cells accompaning with the cell reaction and the fibrin exudation in abdominal cavity, occurs spontaneously after 8–12 days during proliferation of tumor cells, and it bases upon the interaction between immune body developed in animals against tumor cells during their growth and tumor cells as a antigen. When the proliferative activity of cells is weak and the development of immune body is strong, the natural healing occurs suddenly, while in inverse condition the animals die of tumor leaving no immunity. Therefore the correlative condition between proliferativity of tumor cells and the development of immune body exerts an important influence upon the death of tumor and the natural healing.

The animals healed naturally from tumor inhibit the retransplantation of the same tumor in a long time (about 100 days), normal rat which were formerly inoculated with the sera of healed rats inhibit the transplantation of this tumor in a certain grade. This serum injured the sarcoma cells in a form of vacuolar degeneration in vitro, it agglutinated the tumor cells and it acted upon protein of tumor cells in the precipitin reaction and the complement fixation test. But the sera of rats died of tumor showed no activity in vitro, and even when these animals were immunized by means of intracutaneous transplanting and extraction by terns they showed no serum reaction, in spite of proving a certain resistance against retransplantation in these animals in a certain period.

The sera of rabbit and goat immunized against Yoshida sarcoma showed a strong cytotoxic effect not only upon Yoshida sarcoma but also upon rats tissues (species specific immunity).

When the sera were absorbed with erythrocytes of rat, there remained still more agglutinin against nucleated cell of rat and Yoshida sarcoma, just like as the sera of Wistar rat healed from Yoshida sarcoma. Being absorbed with emulsion of nucleated cells of rat or of killed Yoshida sarcoma, the sera showed no serum reaction against rat or tumor cells in vitro, but they had still injurious action upon Yoshida sarcoma in a certain grade in vitro and in vivo, just like as the immune sera of Yamashita rat against Yoshida sarcoma.

When these immunized sera were absorbed sufficiently with raw Yoshida sarcoma, they showed no action not only upon rat but also upon tumor cells.

Wistar rat immunized against normal tissues of Yamashita rat inhibit the transplantation of Yoshida sarcoma in a certain period and its serum showed a "taken" in the agglutination test against normal cells of Yamashita rat and also against Yoshida sarcoma in a slight grade. But when the sera were absorbed with normal cells of Yamashita strain it showed no action on both these normal and pathological cells in vitro or in vivo.

Conclusively it is to say that the immunity against Yoshida sarcoma is partial-

ly depend upon the normal strain specificity between transplanted (immunized) rat strain and the strain from which Yoshida sarcoma generated. But there exists in sera still more an unkown factor which acts specifically upon Yoshida sarcoma.

The immunological therapy on Yoshida sarcoma was also carried out with above mentioned immune sera. When the raw sera of rabbit immunized against Yoshida sarcoma were inoculated in Yamashita or hybrid rat 2 days after tumor transplantation, the tumor cells vanished in cytolysis and disappeared from the cavity in a short time, but most of rats died of serum intoxication, while the application of immune sera absorbed sufficiently with normal rat tissues resulted disappearence of only the tumor cells in cavity, and the rats recovered from tumor completely without intoxication in considerable cases.

The sera of Wistar rat healed from Yoshida sarcoma were also effective for the benefit of complete healing of Yoshida sarcoma when they were used in a plenty dosis.

But it is a problem whether this anti-tumor factor is truely based upon the tumor specific immunity or on a small difference between two strains of rat. Therefore it is a question further more whether this immunological therapy can be successfull on originated tumor or not.

We are now studing further on this problem by means of some other ascites tumors of rat which resemble to Yoshida sarcoma (MTK. & Hirosaki type) and by means of a new ascites tumor of rat which is different from Yoshida type (Takeda sarcoma).

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抄　　錄

吉田肉腫の免疫現象に関する研究

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腫瘍における免疫及びアレルギー反応というデリケートな問題の追求に適当した吉田肉腫及び若干の純系白鼠の系統が提供されたので先ずその累代移植の成績から研究を開始した，その結果，山下系白鼠は吉田肉腫移植ですべて腫瘍死して最も吉田肉腫発生動物系に近い態度を示し，一般市販雑婚白鼠はほぼこれに類した，Wistar 系は初期世代は腫瘍死し後世代ではやや増殖力を減じて常に 10 日前後に腫瘍細胞は突如空胞変性に陥って消失して自然治癒した。どぶ鼠では腫瘍死と自然治癒が混在し，くま鼠，廿日鼠は何れも自然治癒したが累代移殖は可能である。

この突如として起る自然治癒は一方移植動物の系統が腫瘍発生動物から遠縁であるために増殖性が低下する事，動物の老若，血清の自然凝集素の有無，正常反応性の強弱などが関係すると共に他方この腫瘍細胞を抗原として移植動物に抗体が発生し腫瘍細胞と反応してチトトキシンアレルギー性反応が成立する事が関係する事を知った。

即ち移植腫瘍における腫瘍死と自然治癒は腫瘍の増殖性と移植動物における抗体発生の相互関係に基くものである。生体は細菌感染時におけると同様にノルメルギー，アレルギー，アネルギーの3反応状態を示し，抗体過剰でアレルギー状態を示して自然治癒し，抗原過剰でアネルギー状態に陥つて腫瘍死する。

腫瘍の免疫の解明には自然治癒現象が対照になるが，自然治癒例への再移植は 100 日以上阻止され強いアレルギー反応を伴う，その血清は試験管内で 40—160×倍稀釈まで吉田肉腫を自然治癒時同様に空胞変性に陥れ増殖性を失わしめる。この血清を先に正常動物に与えればその後の移植は抑制される。

自然治癒血清は又腫瘍細胞を 80—640×迄凝集し，同時に山下系腹腔単球を凝集するがその赤血球を凝集せず有核細胞に共通する抗原性を認める。腫瘍死鼠血清はしかし凝集素を示さず又山下系等に吉田肉腫の皮下移植剔出をくりかえせば長期間の抗移植性を現すが血清の凝集反応は陰性である。

兎，山羊を吉田肉腫で免疫すれば総ての鼠細胞及び吉田肉腫を凝集融解する強い抗鼠性（種属特異性）のチトトキシンを生ずる。これを鼠の赤血球で吸収すれば腫瘍凝集素はなお残り，

自然治癒鼠血清に近づく，これを正常有核鼠組織で吸収すれば腫瘍凝集素もと0なるがなお生体内外で吉田肉腫を空胞変性させる因子が残り抗移植性を与えた山下系血清に近づく，これを生の吉田肉腫で吸収すれば全く抗肉腫性も失われる。即ち腫瘍免疫は抗鼠性の種属特異性，抗鼠有核細胞特異性の他に吉田肉腫に特に作用する独立した因子が存在する，

事実吉田肉腫発生系統鼠の正常組織でこれと遠縁の白鼠を強く免疫すれば一過性ながら吉田肉腫移植を阻止する弱い免疫を得る，しかしこれを正常組織で吸収すれば抗吉田肉腫性は失われる。

以上の免疫血清によつて吉田肉腫の治療を行うに，Wistar 系移植では何れの免疫血清でも自然治癒は促進される。しかし山下系，市販雑婚白鼠では多くは中毒死，再発腫瘍死を来す，唯 Wistar 系の自然治癒血清の大量，鼠の赤血球で吸収した兎の免疫血清で完全治癒が成立する。また抗吉田肉腫兎血清を種々の操作で正常鼠組織あるいは殺した吉田肉腫で吸収し，その有核無核細胞凝集価の0になったものでは鼠を障害せず完全治療が成立し再発を見なかった。

しかし以上の抗腫瘍因子が直ちに腫瘍特異性を意味するか，また移植腫瘍の免疫治療から直ちに発生腫瘍のそれに及び得るかは今後の問題である。ただ従来の成績に反して移植腫瘍の免疫治療の可能な事実の一つは吉田肉腫が液状の腫瘍で有効物質が個々の細胞に直接作用し得るからである。

我々は以上の研究を更に発展させるためにその後に発生した吉田肉腫と同型の腹水腫瘍 (MTK,$_{1-2}$, 弘前型) 及びわれわれの教室で山下系から発生した吉田肉腫から独立した別種の新腹水腫瘍 (武田肉腫) について免疫の相互関係の研究を続行中である。

本研究は多数の教室研究者の共同研究であり，その研究費は文部省科学研究費の外に Saõ Paulo, Brasil の今田求氏の研究資金でまかなわれた。

BOOK REVIEW

The "Atlas of Tumor Pathology", edited by the Subcommittee on Oncology and for sale through the American Registry of Pathology, Armed Forces Institute of Pathology, Washington, D. C., U.S.A.

Following the suggestion made at the IV International Cancer Congress in 1947, the Committee on Pathology of the National Research Council of the United States formed the Subcommittee on Oncology in the same year to make a renewed attempt to simplify and standardize the nomenclature of the neoplastic diseases, and to device more adequate means for the education of the pathologists and medical personnels. This "Atlas" has been appearing since 1949 as the answer of the Subcommittee to the requests and as the results of continuing coordinate efforts of the American pathologists. Sponsored and supported by the American Cancer Society, National Cancer Institute, Armed Forces Institute of Pathology, U.S. Veterans Administration and two private Funds, the "Atlas" is scheduled to be published under a long-range plan in 39 fascicles of loose-leaf form, including an index. Being a Government publication, and especially under support of the U.S. Armed Forces, each fascicle is obtained at amazingly low price.

It is divided into eleven sections according to the regions and systems of human anatomy. Tumors of the central nervous system are also to be included. By the end of 1951 the following six fascicles were published:

Section II, Fascicle 6: Tumors of the Peripheral Nervous System, by Dr. A.P. Stout, 1949 (57 pp., 56 figg., 60¢).

Section III, Fascicle 9: Teratoma, by Dr. R. A. Willis (England), 1951 (58 pp., 45 figg., 50¢).

Section IV, Fascicle 16: Tumors of the Carotid Body and Related Structures, by Dr. P. M. Le Compte, 1951 (40 pp., 23 figg., 45¢).

Section V, Fascicle 18: Tumors of the Mediastinum, by Dr. H. G. Schlumberger, 1951 (88 pp., 75 figg., 75¢).

Section VIII, Fascicle 29: Tumors of the Adrenal, by Dr. H. T. Karsner, 1950 (60 pp., 47 figg., $1.00).

Section IX, Fascicle 34: Tumors of the Breast, by Dr. F. W. Stewart, 1950 (114 pp., 68 figg., $1.10).

Reviewing the fascicles already published, the original aims of the Subcommittee apear to be almost completely realized in them. A single preferred name is given to each one tumor for the purpose of standardization of nomenclature, but more important synonyms are listed. Pictorial representations of the diseases grouped under the term cancer and of the borderline lesions make the nuclei of

these fascicles. Although each author is an expert in that particular line, materials were contributed from all medical resources of the U.S. Illustrations are printed on special art-papers, large-sized, clear, numerous and excellently chosen. Several color pictures are also reproduced. Clinical and biological characteristics, sometimes including behaviors in vitro, both gross and microscopic morphology of the tumors and border-line lesions with differential criteria, are stated in brief and precise form. Bibliographies are to the points and up-to-date.

Each contributor states in brief explanatory legends what he believes to be the truth and does not engage in lengthy discussion. This makes a sharp contrast to the famous Henke and Lubarsch's Handbook, published after the World War 1 from Germany. When accomplished, this "Atlas" will be one of the best contributions of the American pathologists to the world's medicine. The reviewer pays high respects to the coordinate works in systematized compilation of the materials and to the publication in an easily accessible form in the interest of pathologists all over the world. (Kunio Oota)